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DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

ANNUAL REPORT OF THE METABOLISM BRANCH
SUMMARY OF SIGNIFICANT ACTIVITIES
US NATIONAL CANCER INSTITUTE.
" Report of program activities.
July 1, 1976 through September 30, 1977

The clinical research program of the Metabolism Branch is directed toward two major goals. The first is to define host factors that result in a high incidence of neoplasia. In this area, a broad range of immunological investigations are carried out in patients with malignancy, in patients with immune deficiency diseases and a high incidence of neoplasms as well as in experimental animal systems to determine the nature of immune processes that are of importance in the surveillance against neoplastic cells and to define the nature of defects in the immunological system that result in an increased incidence of neoplasia. Major efforts in this area are also directed toward: 1) Defining events of cellular maturation, cellular interaction and cellular biosynthesis involved in the normal circulating antibody response. Specific emphasis is placed on studies of immunoregulatory cells including helper T cells, suppressor T cells and macrophages that regulate antibody responses and on studies of disorders of these immunoregulatory cell interactions in patients with immune dysfunction. 2) The genetic control of the immune response especially as related to the immune response genes associated with the major histocompatability complex. 3) Studies of the mechanism of action of various cytotoxic cells and their contribution to host defense. 4) Detection of circulating tumor associated antigens. The second major goal of the Branch is to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. Both patients with neoplastic disease as well as those with non-neoplastic disorders that facilitate the development of techniques for the study of cell membranes, homeostatic mechanisms and metabolic derrangements of biochemical control mechanisms are being investigated. Within this area a special emphasis is laid on the biochemical events accompanying normal growth and the hormonal control of this growth as it relates to our understanding of malignant growth.

Immunoregulatory Cell Interactions in Immune Response

A major effort of the Metabolism Branch over the past few years has been directed toward defining the major events of cellular differentiation, cellular interaction and cellular biosynthesis involved in the specific circulating immune response. These studies have placed special emphasis on defining the defects of regulatory T cell and macrophage interaction with B lymphocytes and plasma cells that occur in patients with primary immunodeficiency diseases associated with a high incidence of malignancy, in patients with autoimmune disorders as well as in patients with malignancies of the T or B lymphocyte systems. Overall these studies were directed at defining the factors in normal and abnormal states controlling the production of antibodies and the synthesis of immunoglobulin molecules. Cells that ultimately produce antibodies undergo sequential maturation from stem cells in the marrow to B lymphocytes and then from B lymphocytes into immunoglobulin synthesizing plasma cells. The maturation of B cells into plasma cells is carefully regulated both positively and

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negatively by a series of separate cells. Specifically many antigens and mitogens require the presence of both helper lymphocytes of thymic origin (helper T cells) and macrophages as well as the B cells to induce a full antibody response. More recently it has been recognized that a separate class of thymic derived cells, suppressor T cells, may act as negative regulatory of B cell maturation inhibiting this process. To study these events we have developed a series of techniques including techniques to study the terminal differentiation of B cells into immunoglobulin synthesizing and secreting cells, techniques to assess helper T cell function, to assess the macrophage contribution to these events and to detect both increased and decreased functional activities of suppressor T cells. In the basic procedure peripheral blood lymphocytes are cultured with polyclonal activators of B cells in vitro and the immunoglobulin synthesized and secreted by these cells is quantitated using radioimmunoassay procedures. The first agent that we used in these procedures, pokeweed mitogen, is a prototype of a thymic and macrophage dependent activator of B cells. When pokeweed mitogen was added to unseparated mononuclear cells from the peripheral blood of normal individuals they stimulated B lymphocytes to mature into plasma cells which synthesize and secrete immunoglobulin molecules. However, when the B cells and macrophages were depleted of T cells they synthesized virtually no immunoglobulin molecules. When normal T cells were added to these B cells, they restored immunoglobulin synthesis to within the normal range. To determine if a population of cells had helper functional activity, they were co-cultured with the purified B cells and macrophages and the synthesis of immunoglobulins was detected. Pokeweed mitogen stimulation was also shown to be macrophage dependent. That is if B cells and T cells were exposed to pokeweed mitogen in the absence of macrophages, no immunoglobulin synthesis ensued. Indeed there was the generation of suppressor T cells. These studies support the view that macrophage presentation of antigens and mitogens to B cells is required for a normal immune response. Over the past year a new promising polyclonal activator of B cell maturation, an extract of Nocardia opaca has been utilized that appears to be less thymic dependent. Thus, we have developed both a mimic of thymic dependent antigens in pokeweed mitogen and of thymic independent antigens in Nocardia opaca. Finally, techniques for the study of deficiency and excesses of suppressor cell action have been developed.

Over the past year a series of studies were initiated to determine whether homogenous populations of T cells from patients with T cell leukemias retain the capacity to help or suppress the synthesis of immunoglobulins from lymphocytes derived from normal individuals. It has hoped that the study of the monoclonal proliferations of malignant T cells and their products may prove as exciting in answering questions regarding cellular immunity and T cell regulation of humoral immune responses as myeloma cells and their paraproteins have proved to be in answering questions concerning humoral immunity. The initial studies in this area focused on patients with the Sézary syndrome. This syndrome is a life-threatening disease characterized by exfolative erythroderma, generalized lymphodenopathy and circulating neoplastic T cells. Lymphocytes from patients with the Sézary syndrome and high numbers of circulating neoplastic T cells synthesized essentially no immunoglobulin in pokeweed mitogen stimulated cultures in vitro. In addition, lymphocytes from the patients with the Sézary syndrome possessed no suppressor capacity. However, the circulating lymphocytes from approximately 70% of the patients with the

Sézary syndrome studied functioned as helper T cells. There was a six to over 30-fold augmentation of immunoglobulin production by normal purified B cells after the addition of Sézary cells. These studies are in accord with our view that the Sézary syndrome in many cases represents a homogenous malignant expansion of helper T cells.

Patients with acute lymphocytic T cell laukemia present with a different pattern from those with the Sézary syndrome. The T cells from these patients with acute T cell leukemia did not function as helper cells in co-culture with normal B cells. However, in one of the four cases studied, the cells of the patient suppressed immunoglobulin synthesis by co-cultured normal cells in the presence of pokeweed mitogen. These cells were not cytotoxic to normal cells nor did they use up required media components by overgrowth of the normal cells. Thus, this patient appears to have a leukemia of the suppressor T cell population or of a population of T cells that can activate precursor cells from the co-cultured normal population to become suppressor T cells.

The majority of patients with the primary immunodeficiency states and immunoglobulin deficiency do not have a lack of helper T cells as their fundamental defect. However, we have demonstrated two children with thymic deficiency states that provide an interesting exception to this generalization. In these two children with associated severe thymic deficiency there was a markedly reduced immunoglobulin synthesis as well. When the lymphocytes were co-cultured with normal T cells or T cells from patients with the Sézary T cell leukemia, they synthesized large quantities of immunoglobulin molecules. Thus, in rare circumstances an isolated defect in helper T cells may lead to immunoglobulin and antibody deficiency even when a normal B cell-plasma cell system is present. For such patients appropriate therapy would be the transplantation of co-cultured epithelial thymus.

When the techniques of immunoglobulin biosynthesis were applied to the study of patients with common variable hypogammaglobulinemia, the patients were shown to be heterogenous with a variety of different immunological defects. The majority of the patients had a primary defect of the B cell plasma cell system. In some cases this was associated with the secondary development of suppressor T cells. In a small percentage of the patients there was a primary defect of suppressor cells that prevented B cells from maturing into plasma cells. Such a development was noted especially in individuals who developed immunodeficiency following infectious mononucleosis. The nature of the defect in patients with selective IgA deficiency was also investigated using in vitro techniques to study pokeweed mitogen driven terminal maturation of B lymphocytes into immunoglobulin synthesizing and secreting cells. Two patterns emerged from these pateint studies. In the majority of the patients there was an intrinsic defect of the B cell-plasma cell series that normally developed into IgA producing cells. In a second group of patients, however, in addition to the defect in the B cells and plasma cells there was an increase in the number or state of activation of IgA class specific suppressor cells that prevent B cells from maturing into IgA synthesizing and secreting cells.

Studies of the immunoregulatory role of T cells have been continued in an animal model of humoral immunodeficiency. In the chicken, a central lymphoid organ, the Bursa of Fabricus is the site of initial differentiation and development of B lymphocytes. If the Bursa is removed in the newly hatched chicken before B cells have emigrated to peripheral lymphoid organs, the chicken develops agammaglobulinemia. This immune deficiency state is characterized by total absence of B lymphocytes by absence of immunoglobulins from the serum and secretions, and by a failure of the lymphoid tissues to develop the characteristic components associated with the system of humoral immunity. T lymphocyte differentiation and function is not deficient in these bursectomized agammaglobulinemic chickens. However T cells obtained from these birds were found to exert a profound effect on the humoral immune system when they were transplanted into normal birds. Recipients of T cell grafts from agammaglobulinemia donors were found to have impaired antibody responses and to frequently develop agammaglobulinemia themselves. This transplantatle or "infectious agammaglobulinemia" was found to be caused by suppressor T cells present in the transplanted cells from the original bursectomized donor. The immunodeficiency which developed in the recipient chickens was characterized by total absence of IgG, IgA, and IgM just as in the bursectomized donors, but differed from donor birds in that the recipient chickens had the persistence of B lymphocytes and germinal centers in their lymphoid tissues and continued to have a Bursa of Fabricus. Thus, these recipient chickens had developed an immunodeficiency state closely paralleling that seen in many patients with common variable hypogammaglobulinemia who also have persistence of B lymphocytes and evidence for excessive "suppressor T cell" activity.

Further study of these birds with "infectious agammaglobulinemia" demonstrated that the suppressor T cells develop in the donor birds after 10 weeks of age and are not in detectable quantities before that time. Immunoglobulin production in the recipient birds ceased almost immediately after injection with the suppressor cell population suggesting that these cells may be exerting an effect directly on the immunoglobulin producing plasma cells. B lymphocytes are initially present in normal numbers in the recipient birds but eventually fall to low numbers further suggesting that the suppressor cell may also act directly on B cells or their precursors.

Dysgammaglobulinemia in man is a disorder characterized by the deficiency of one or more classes of immunoglobulin with persistence of normal levels of the other immunoglobulins. When T cells from various agammaglobulinemic chickens were transplanted to normal recipients in limiting dilution assays, recipient birds were found to develop dysgammaglobulinemia with selective loss of only one class of immunoglobulin. The deficiency which ultimately developed in the recipient birds varied from donor to donor suggesting that suppressor cells for each individual class of immunoglobulin are present in the donors and that the predominant suppressor cell class is revealed by the limiting dilution assay. Antibody responses in the dysgammaglobulinemic birds were present in the non-suppressed Ig classes and as in the birds which developed agammaglobulinemia following transplantation, B cells continued to be present bearing the immunoglobulin of the suppressed Ig class. This Ig class specific suppression also closely parallels immune deficiency disorders seen in man and provides an excellent experimental model to further investigate the role

of T cell regulation of Ig production and may allow the development of therapeutic approaches for the correction of such deficiencies in man.

In the previous studies the disordered suppressor cells was of the T cell series. Patients with multiple myeloma appear to have a different defect as the cause of their significant reduction in serum levels of polyclonal immunoglobulins and in the capacity to synthesize antibody in response to antigenic challenge. These patients have markedly reduced polyclonal immunoglobulin synthesis in vitro. In addition, in co-culture with normal cells they have a suppressor. However, in contrast to the observations in patients with common variable hypogammaglobulinemia or thymoma and hypogammaglobulinemia the cell appears to be a phagocytic mononuclear cell. Over the past year using an experimental model of transplantable myeloma in mice, we have demonstrated that the suppressor cell is a monocyte. Thus, both in the animal model and in man the hypogammaglobulinemia is due in part to the activation of immunoregulatory macrophage-like cells.

As discussed above excessive numbers of activated suppressor T cells may be associated with hypogammaglobulinemia and other forms of immunodeficiency. However, it should be noted that such cells normally play a key role in the negative control of the immune system in general and in the development of specific immunological tolerance. We have used an animal modal system of autoimmunity, that of the NZB/W mice to analyze this question. These mice develop an autoimmune complex similar to that observed in patients with systemic lupus erythematosus. To approach the question of T cell suppressor function in NZB/W mice more directly we have taken advantage of the observation that splenic T cells can be activated by Concanavalin A to become cells which suppress the immunological function of other cells. Using this system we have shown that the NZB/W mice lose precursors of suppressor T cells as they age. In addition, they lose the capacity to secrete a normal physiological suppressor protein, soluble immune response suppressor. Although these animals cannot generate negative signals they can respond effectively to suppressor T cells generated from normal spleens and to the soluble immune response suppressor derived from normal T cells. When NZB/W mice were treated with SIRS from normal animals from an early age, they developed hypogammaglobulinemia as well as the abrogation of all manifestations of autoimmunity including the development of anti-DNA antibodies, Coomb's positive hemolytic anemia and immune complex glomerulonephritis. In addition, 90% of treated animals were alive after one year of treatment, whereas in the control group, only 10% of the animals were surviving at this time. Further studies are underway to find the active factor in the Concanavalin A supernatants, to determine whether established disease can be reversed by SIRS treatment and to determine whether patients with autoimmunity also lack suppressor T cell function.

As part of the studies in man a system of autoreactivity present in normal animals is being investigated. A striking example of autoreactivity present in normal animals and man is the capacity of T lymphocytes to respond to B lymphocytes from the same individual. We have found that in vitro, this response is demonstrated as an autologous mixed lymphocyte reaction (MLR) when T lymphocytes are induced to undergo vigorous proliferation when exposed to autologous B lymphocytes. A variety of substances with immunoregulatory properties were studied for their effects on this form of lymphocyte

autoreactivity. At physiological and supraphysiological concentrations of alpha-fetoprotein and the thymic hormone thymosin, no detectable effect was observed on the autologous MLR. Cortisol at pharmacologic concentrations has been shown to be suppressive to a variety of *in vivo* and *in vitro* lymphocyte functions. We found that at such concentrations *in vitro*, hydrocortisone had moderate suppressive effects on the allogenic MLR and surprisingly totally abolished all autoreactivity as detected in the autologous MLR between T and B lymphocytes. This differential sensitivity of autoreactive T cells was found to be maintained even at physiologic concentrations of cortisol. Under these conditions, reactivity to allogeneic cells was completely unaffected while autoreactivity was inhibited by 98-100%. Thus, concentrations of cortisol found normally in human plasma have a profound immunoregulatory role in selectively blocking autoreactivity of at least one class of immunocompetent T cells.

Genetic Control of the Immune Response

Discovery in the last decade of the existence of immune response (*Ir*) genes, which control the ability of an animal to respond to an antigen with a high degree of antigenic specificity, has opened the door to a potential understanding of the regulatory factors which control the immune surveillance against malignancy and infectious disease as well as the abnormal immune responses involved in the pathogenesis of autoimmune and other diseases. Most *Ir* genes studied have been those controlling the response to relatively simple synthetic antigens and hapten-carrier complexes. Over the past two years, a study in our laboratory of the fine specificity of *Ir*-gene control of the antibody response to a natural protein antigen, staphylococcal nuclease, in mice, has shown that the control by *H-2* (major histocompatability complex or MHC) genes can be exerted at the level of individual antigenic determinants rather than the whole antigen molecule. This result disproves the generally accepted notion that many of the MHC-linked *Ir* genes act through recognition by helper T cells of a "carrier" portion of the antigen molecule which would allow response to the whole molecule. In collaboration with Drs. David Pietsky and David Sachs (Immunology Branch, NCI) we have now extended these studies to show their general applicability in other congenic mouse strains. We have also shown that an additional gene (or genes) linked to neither *H-2* nor heavy chain immunoglobulin allotype (structural genes) is also involved in the control of the response to nuclease. The non-*H-2*-linked gene(s) controls the overall level of response to the whole molecule which can be reached after multiple immunizations, while the *H-2* linked genes control the proportions of antibodies made to different determinants. In collaboration with Dr. R.H. Schwartz (Lab of Immunology, NIAID), we have further studied this control in a purely T cell proliferative response. Similar control appears to apply, and more than one *H-2*-linked *Ir* gene is involved in controlling the response to the several determinants of nuclease. These T cell proliferative responses can be blocked by anti-Ia antisera directed at antigens coded in specific subregions of the *I* region of the *H-2* complex.

In order to find another antigen system with more flexibility with which to extend these studies, the antibody response to mammalian myoglobins in mice was studied for the possibility of *H-2*-linked control. Definitive evidence

has been gathered to demonstrate the existence of clear H-2-linked Ir gene control of the response, as well as control by other non-H-2-linked genes. In addition, evidence strongly suggests the existence of at least two different Ir genes mapping in different I subregions of the H-2 complex both involved in the response. Thus, this myoglobin system promises to be a useful tool in the study of the mechanism of genetic control of the immune response.

Gluten Sensitive Enteropathy

A major example of a human disease wherein a disordered immune response is related to genes associated with the major histocompatability complex as well as to genes on a separate chromosome. GSE is a disease of the GI tract associated with malabsorption, villous flattening, inflammatory cell infiltration of the GI tract and an increased incidence of neoplasia. It is due to the toxic effect of wheat proteins (gluten) on the gastrointestinal mucosa. In previous studies we observed that approximately 80% of patients with GSE bear the HLA-B8 histocompatability antigen. In current studies we have determined that GSE is associated with a high incidence of a second histocompatability antigen, HLA-DW3. This antigen is detected with appropriately absorbed antisera used in conjunction with a cytotoxicity assay in which the target cells are peripheral blood B-cells isolated with the use of anti-FAB coated microtitre plates. We have found that approximately 80% of GSE patients bear HLA-DW3 in contrast to 35% of random control individuals. HLA-DW3-positivity was found among both HLA-B8 positive and HLA-B8 negative individuals.

At this point in our knowledge it is not clear whether the HLA genes responsible for the antigens associated with GSE take a direct part in the disease mechanism underlying GSE or, alternatively, are linked to an as yet unidentified gene, which is the true "GSE gene." Evidence for the former possibility was obtained during this period in a study done in collaboration with Dr. Z. M. Falchuk at the Peter Bent Brigham Hospital. We found that patient responses in in vitro organ culture depended on HLA status. Specifically, in a prospective study of patients with diarrheal illness tissue obtained from 23 GSE patients were subjected to organ culture in the presence and absence of gluten-protein (peptic-tryptic digest of gluten). Nineteen of the 23 patients demonstrated gluten toxicity in organ culture (i.e., failure to undergo epithelial cell maturation); all 19 of these patients were HLA-B8 positive. The four remaining patients failed to demonstrate gluten toxicity in organ culture; three out of four of these patients were HLA-B8 negative. In companion studies using purified gliadin in the organ culture system it was observed that 10 of 11 HLA-B8 positive patients demonstrated gluten toxicity in organ culture whereas seven of eight HLA-B8 negative patients failed to demonstrate gluten toxicity in organ culture. These studies point to the conclusion that "HLA-B8 status" in some measure determines the degree of toxicity to gluten protein. This is likely to be due to the fact that the cell surface configuration which recognizes gluten on the epithelial cell is made up, at least in part, by the histocompatability antigen.

It has been clear, as a result of analysis of the inheritance of GSE in families studied in relation to the inheritance of HLA antigens, that HLA antigens cannot be the only genetic determinants important to the occurrence of GSE. In this regard, we have recently discovered a second genetically determined cell surface protein that is associated with GSE, the GSE-associated B cell antigen. This specificity is found on peripheral B cells (but not T cells) of patients and is detected with antisera from mothers of patients who were presumably immunized with patient cells during pregnancy. Using a cytotoxicity test specific for B cells we determined that the B cell antigen is present in 90% of patients, but only 20% of normals. Since the sources of the antisera, the mothers of patients, were themselves HLA-DW3 positive and, further since some patients and controls were discordant for GSE-associated B cell antigens and HLA-DW3 it is likely that the GSE-associated B cell antigens and HLA-DW3 represent separate specificities.

Using the typing methods for HLA-DW3 and GSE-associated B cell antigens alluded to above, individuals in seven families of patients with GSE were characterized by antigen type. As a result, several conclusions concerning the genetic background of GSE can now be drawn: 1) It was found that all parents of patients were positive for the B cell antigen since only 20% of random individuals are positive the high degree of positivity among parents strongly suggests that a pre-condition for the occurrence of GSE is the inheritance of the appropriate B cell antigen from both parents; in other words, patients were homozygous for the GSE-associated B cell antigen; 2) most patients were positive for HLA-DW3 as well as the GSE-associated B cell antigen; it is therefore likely that at least a single dose of the gene controlling HLA-DW3 (or a related specificity) is necessary for the occurrence of GSE; 3) the inheritance of the GSE-associated B cell antigen and HLA antigen was discordant, that is in several families siblings were found who were GSE-associated B cell antigen identical, but HLA non-identical. These studies, in summary, indicate that GSE is controlled, at least in part, by two non-linked genes, one in the major histocompatibility locus and one on a separate chromosome not containing the major histocompatibility locus.

The foregoing genetic study should not be construed as indicating that the genes identified are the only genetic or environmental antecedents of GSE. In this period we have studied a set of twins, one with GSE and one without GSE. This twin pair were proven identical on the basis of at least 6 different polymorphic systems including red cell isoenzymes and surface antigens, B cell antigens, HLA antigens and fingerprint studies. This discordancy in this identical twin pair gives rise to the expectation that environmental factors will ultimately be found to influence the occurrence of GSE.

During this period we have initiated studies on the nature of the association between B cell antigens and GSE. In this regard, an important observation has been that the maternal antiserums recognizing the GSE-associated B cell antigen fails to induce lymphocyte cytotoxicity when cells from patients are pre-incubated with gluten proteins. This observation implies that the GSE-associated B cell antigen is a receptor molecule for gluten protein and as such, is important in the initiation of the immune responses which are central to the pathogenetic mechanism underlying GSE.

Mechanism of Action of Cytotoxic Mononuclear Cells and Their Contribution to Host Defense

Cytotoxic killer cells have been implicated in a number of systems of host defense including the destruction of malignant cells. The studies of the types of cells involved and the mechanism of action of these various cytotoxic killer cells and their contribution to host defense were continued. Model systems have been developed for the study of killer T cells, cytotoxic K lymphocytes and cytotoxic monocytes in a series of distinct cytotoxic processes. These have included antibody dependent cellular cytotoxicity (ADCC), mitogen induced cellular cytotoxicity (MICC), natural killer or natural cytotoxicity and spontaneous cell mediated cytotoxicity (SCMC) and monocyte dependent cytotoxicity. Antibody dependent cellular cytotoxicity (ADCC) is a form of cell mediated killing in which non-immune effector cells attack and kill target cells sensitized with IgG antibody. Mitogen induced cellular cytotoxicity (MICC) is a form of cell-mediated killing in which non-immune effector cells kill target cells in the presence of a plant mitogen or lectin. Natural cytotoxicity or spontaneous cell-mediated cytotoxicity (SCMC) represents the destruction of a variety of cells in vitro by a population of cells that had not been previously immunized. Both MICC and ADCC are surface mediated killing events which do not require phagocytosis of the target cells. When chicken erythrocytes were employed in assays of MICC, and ADCC a variety of cell populations mediated could affect cytolysis, whereas when metabolically active target cells such as those of the Chang liver were employed, MICC was shown to be a T cell mediated and ADCC a K cell mediated event. The mechanism of these surface mediated cytotoxicity reactions ha.. been obscure. Inhibitors of effector cell DNA, RNA and protein synthesis have not in general reduced the cytotoxic capacity of these cells. Ouabain, a cardiac glycocyte functions as an inhibitor of NAK and Ca++ membrane transport systems. The addition of ouabain to MICC and ADCC reactions inhibited MICC by greater than 95% while inhibiting ADCC by approximately 50%. EDTA and EGRA, chelating agents which bind calcium also inhibited MICC reactions to a significantly greater extent than ADCC. The inhibitory effects of these agents on MICC reactions could be completely reversed by the addition of calcium to the cell cultures. These data suggest a critical role for calcium ion flux in their cytotoxic action of certain effector cell populations and helps our understanding of basic mechanisms by which effector cells mediate their killer potential.

Additional studies were undertaken to examine the phenomenon of spontaneous cell-mediated cytotoxicity which has been correlated with tumor resistence and which may play a significant effector cell role in immune surveillance. We have established an assay for SCMC for human cells employing Chang cell targets. The effector cell mediating SCMC like the effector mediating ADCC was shown to be in the K cell population. That is, a population of lymphocytes that do not bear immunoglobulins on their surface as do B cells and do not rosetts with erythrocytes as do T cells. The mechanism of cytotoxicity for SCMC was demonstrated to be different than that of ADCC in that SCMC was inhibited by trypsin pretreatment of effector cells and this inhibition was not reversible by incubation in autologous plasma. Thus, although cytolysis in both assays is mediated by K cells, SCMC and ADCC were whown to involve separate cytotoxic mechanisms which for SCMC probably does not involve

arming in vivo with antibodies.

Work is currently underway in a new model to examine spontaneous in vitro generation of a potent class of specific cytotoxic monocytes. The mechanism by which these cells develop cytotoxic potential is dependent upon a time dependent loss in vitro of suppressor cells that normally inhibit this form of cytotoxicity. Thus, freshly drawn mononuclear cells when assayed are not cytotoxic. However, after seven days of in vitro culture in the absence of any known exogenous stimulation the monocytes in these cultures develop profound nonspecific cytotoxicity towards all types of red blood cell targets tested. The addition of autologous freshly drawn lymphocytes to these seven day cultures suppresses killing to baseline levels. The nature and mechanism of action of the suppressor cell is under active investigation. It appears to be a non-T cell. Furthermore, sonicated supernatants of freshly drawn cells contain a heat labile suppressor factor. Our expanding interest in this unique model stems from several observations. First, the model allows a potentially fruitful approach to study the negative regulatory influences which control cytotoxic monocyte function in vitro and in vivo. Second, this type of antigen nonspecific non-immune monocyte killing is quite analogous to natural killing systems involving K cells and evidence is accumulating from numerous labs that this type of early nonspecific killing may be a very important first line of defense against both infections and malignant diseases. Third, we have discovered that our spontaneous killing model varies immensely with various disease states. For example, patients with acute viremia and certain patients with lymphomas kill without the necessity of preculturing. Our data suggests that these patients lack circulating suppressors. Other patients, however, lack the ability to kill. Patients with CLL cannot kill due to a lack of circulating monocytes. The cells of patients with the Wiskott-Aldrich syndrome cannot kill. This is an independent confirmation of the previously described monocyte killing defect. Certain immunodeficient patients including a subset of common variable hypogammaglobulinemic patients and all patients with ataxia telangiectasia fail to kill and the reasons for this deficiency are under active investigation.

Detection of Circulating Tumor Associated Antigens

The studies using sensitive radioimmunoassays for the oncofetal proteins alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) have continued. Over the past year a new assay for radioimmunoassay for alpha-fetoprotein has been established that significantly extends the lower limit of detectability of this protein. There are virtually no elevations of alpha-fetoprotein in benign conditions and physiological states outside of pregnancy, the disease ataxia telangiectasia and disorders associated with extreme hepatocellular regeneration. In extension of our previous studies we have found that 79 of 80 patients with ataxia telangiectasia have an elevated alpha-fetoprotein level whereas no other immunodeficiency or neurological state has such a defect. This retention of an oncofetal protein in this disorder supports the view that there is a failure of differentiation and maturation for those systems that require the interaction of an entodermal analage with a mesenchymal inducer.

The combined markers alpha-fetoprotein and human chorionic gonadotropin are clearly of great value as markers for following the therapy of hepatocellular tumors, germ cell tumors of the testis other than semonomas. There have been no false positive values utilizing these markers and only 10% false negative values. It has clearly been shown over the past year that alpha-fetoprotein and human chorionic gonadotropin are produced by separate cells in the testicular tumors and that one may have a discordance of these markers during chemotherapy when there is a difference in the response of the different tumor elements to therapy. Thus, it is well established that both markers should be measured at the time of diagnosis and that both markers should be followed during patient therapy.

Proline Transport, Biosynthesis and Metabolism

In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1-pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminates ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps remain undefined in mammalian cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase. Hydroxyproline degradation follows a scheme analogous to that for proline. Hydroxyproline is converted first to 3-OH-PC and then to γ-OH glutamate.

Proline oxidase, the enzyme which catalyzes the first step in proline degradation, has been emphasized during the past year. The enzyme is tightly bound to mitochondrial membranes and the protons generated by proline oxidation are directly transfereed to cytochrome C of the electron transport chain. In addition, the carbons from proline degradation directly contributes to the TCA cycle and carbohydrates. Due to these considerations, proline oxidase and its regulation is of major importance.

We first demonstrated that proline oxidase is localized to certain tissues. In the order of decreasing specific activities, liver, heart, kidney, and intestinal mucosa have proline oxidase. In contrast a variety of other tissues have no detectable proline oxidase. These include: skeletal muscle, circulating blood cells, cartilage (epiphyseal, xiphoid, and costochondral) membranous and diaphyseal bone and skin.

Glucose production from proline was directly shown using in vitro incubations of rat liver minces. We determined the production of both total glucose and glucose-^{14}C from precursor proline-^{14}C. After an initial 10 minute delay, accumulation of glucose in the media was linear for 60 minutes. With increasing media proline concentration glucose accumulation increased but plateaued with proline at 5 mM. Relative to glucose production the half-maximal concentration for proline was about 2.5 mM, a concentration similar to the Km of proline oxidase for proline. At saturating concentrations of amino acid precursors, glucose production from proline was about 50% of that from alanine, a well-known amino acid source for gluconeogenesis. These studies clearly show that livers can utilize proline as a significant source for gluconeogenesis.

Since mammalian organisms have a number of sources for metabolic fuel, it would be advantageous to regulate amino acid degradation when alternative fuel sources are available. With high rates of glycolysis (e.g., exercise) lactate formed and released by liver and converted back into glucose. We found that lactate reversibly and specifically inhibits proline oxidase, thus, linking the rate of proline degradation to carbohydrate metabolism. Inhibition of proline oxidase increases with increasing lactate concentration. With proline concentration at 0.1 mM, lactate at 5 mM inhibits 90% of the enzyme activity. The lactate effect is specific to proline oxidase. Lactate at 5 mM was without effect on a number of other cytosol and mitochondrial enzymes. The lactate effect was also specific in that inhibition was not generalizable to other metabolic intermediates. Besides lactate, only pyruvate and succinate inhibited proline oxidase. The modest inhibition by pyruvate and succinate, however, was seen only a supraphysiologic concentrations. A variety of other intermediates (alpha-ketoglutarate, glucose, glutamate, fumarate, oxalacetate, acetoacetate, acetyl-CoA, B-hydroxy butyrate, alpha-ketoisocaproate) were without effect. Inhibition of proline oxidase by lactate was completely reversible. After exposure to lactate at 37° for 30 minutes, mitochondria recovered by washing and centrifugation had full enzyme activity. More importantly, enzyme solubilized using Triton X-100 was inhibited by lactate, thus, ruling out interference by lactate of proline entry into mitochondria as the basis for the inhibitory effect. Kinetic studies showed that lactate increased the Km of proline oxidase for substrate proline but was without effect on enzyme Vmax. Kinetic studies with lactate at a variety of concentrations showed that the Km of proline oxidase for proline increased linearly as a function of lactate concentration.

These in vitro observations were corroborated by previously published observations in man were hyperprolinemia has been associated with genetic and acquired lactic acidosis. Our in vitro observations may be the basis for this previously unexplained metabolic derangement. Furthermore, the regulation of proline oxidation by lactate may serve as a model for control of gluconeogenesis and metabolic fuel allocation.

Mechanism of Action of Growth Hormone

An understanding of the biochemical events accompanying normal growth (as mediated by growth hormone, for example) may be a prerequisite for understanding malignant growth. There is evidence that the anabolic action of growth hormone is mediated by a second protein factor designated somatomedin. Part of the laboratory effort has continued to be directed toward the purification of a somatomedin-like polypeptide (multiplication stimulating activity or MSA) from the conditioned medium of a rat liver cell line that multiplies continuously without serum. This polypeptide has now been purified to homogeneity and the amino acid composition determined.

It was originally proposed that the rat liver cell line that produces MSA is able to multiply without serum because it produces its own growth factor (MSA). We have recently shown, however, that two other rat liver cell lines (one a subclone of the producer line) are also able to multiply in serum-free medium but not produce MSA.

A radioimmunoassay for MSA has recently been developed in our laboratory and studies are underway to study the physiology of MSA in the rat including site of synthesis and circulating levels.

MSA-receptor studies have continued in collaboration with Matthew Rechler (NIAMDD). Extensive cross reactivity has been demonstrated between MSA and somatomedin A and NSILA-s (nonsuppressible insulin-like activity). Thus, MSA appears to be related to the human somatomedins. In additional cell receptor studies, performed in collaboration with George Todaro (NCI), MSA has been shown to bind to a wide variety of cells in tissue culture, including cell lines derived from human cancers.

Using labeled MSA as radioligand we have recently demonstrated a specific somatomedin binding protein in rat serum. Furthermore, we have produced evidence that the binding protein is under growth hormone control. That is to say, the serum concentration of the binding protein appears to be low in growth hormone deficient rats and to be corrected toward normal by growth hormone treatment of the growth hormone deficient rat. These findings supplement earlier studies from our laboratory showing that the serum half-life of somatomedin is under growth hormone control.

Mutant dwarf mice may be useful in studies directed at understanding the biochemistry of normal growth. We have recently measured serum somatomedin levels in several mutants. The Snell dwarf mouse (dw/dw), the Ames dwarf (df/df) and the little mouse (lit/lit) were found to have low somatomedin levels, whereas normal levels were found in the pygmy mouse (pg/pg). The finding of normal somatomedin levels in the pygmy mouse, a mutant with growth hormone resistance, raises the possibility that this mouse may have an abnormal somatomedin receptor.

Porphyrin Metabolism

The studies of experimental porphyria and the human disease, acute intermittent porphyria and the studies of the control mechanisms of porphyrin and heme biosynthesis have been extended. ALA synthetase is the rate controlling enzyme of heme biosynthesis. It's synthesis is repressed by hemin in vivo. It was concluded that if symptoms of acute intermittent porphyria (AIP) were due to excess of porphobilinogen, aminolevulinic acid or substances derived from them, hemin infusions should result in a fall in the production of these substances and in turn improve the symptoms. Previously a patient who has died from an attack of acute porphyria was studied here with hemin administration on an emergency basis. It was shown that hemin in the doses utilized cured the biochemical abnormalities of the disease and that porphyrin precursor production was reduced to normal. Over the past year the effect of hematin on the biochemical and clinical manifestations of acute intermittent porphyria was investigated by the administration of 19 courses of hematin (defined as 3 mg/kg of hematin intravenously daily for a period of 3-7 days) to 11 patients. In all patients intravenous hematin led to a decline of urinary aminolevulinic acid and porphobilinogen excretion. Values of these porphyrin precursors could be decreased to normal levels by continued therapy

at doses which had no side effects and no evidence of toxicity. However, established motor neuropathy was not dramatically affected.

Hemopexin levels in blood have been shown to be increased in patients with acute intermittent porphyria and to increase further during acute attacks of the disease. Hematin therapy lowered blood hemopexin to unmeasurable levels. Finally, intravenous administered hematin was shown to function as the prosthetic group of tryptophan pyrolase in liver in addition to its capacity to act in the respresion of hepatic ALA synthetase. These studies taken as a whole suggest that the intravneous administration of hematin may be of significant value in aborting acute attacks of intermittent porphyria.

A number of new clinical features have been observed in a number of patients with acute intermittent porphyria. Hyperamylaseminia was shown to be associated with acute intermittent porphyria in 4 patients. In one patient this was macroamylase. Chemical manifestations of hyperthyroidism were observed in 10 patients with acute intermittent porphyria, two of these patients required therapy for clinically evident hyperthyroidism.

Another red cell enzyme test for acute intermittent porphyria was developed. It measures uroporphyrin I synthetase activity, the enzyme that is deficient in patients with acute intermittent porphyria, by spectrophotometric rather then fluorometric method and utilizes the cheaper and more readily available delta aminolevulinic acid rather than porphobilinogen as a substrate. This technique is easier for clinical laboratories to perform than the previously described fluorometric technique. Family studies for the correlation of red cell enzyme defect of acute intermittent porphyria and the abnormal clinical findings in the urine show that a significant number of afflicted individuals cannot be detected by urinary testing but reveal their abnormality when red cell enzyme is investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4002(c)-09-MET

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Defects in Immunoregulatory Cell Interactions in Patients With Immune Dysfunction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas A. Waldmann, M.D.	Senior Investigator	MET	NCI
OTHER:	Samuel Broder, M.D.	Investigator	MET	NCI
	K. Robert McIntire, M.D.	Senior Investigator	LID	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were directed toward determining the steps involved in the differentiation of B cells into immunoglobulin synthesizing and secreting plasma cells. Special emphasis was laid on developing new techniques to study the role of helper T cells, macrophages and suppressor cells in these immune regulatory processes and to define defects in these immunoregulatory cell interactions in patients with immune dysfunctions. Leukemias of both suppressor and helper T cells have been identified. Excessive numbers of suppressor T cells have been demonstrated in association with agammaglobulinemia and selective IgA deficiency. Excessive numbers of non-T cell suppressors have been demonstrated in multiple myeloma associated with immunodeficiency. Finally a deficiency of this immunoregulatory system has been demonstrated in animal models of auto-immunity. Developments of techniques for detection of circulating tumor-related antigens has also been an objective of this overall study.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of the study were: to determine the sequential steps involved in the differentiation of lymphocyte B cells into immunoglobulin synthesizing and secreting cells. Major efforts were directed toward defining the role of suppressor T lymphocytes, helper T lymphocytes, and macrophages in this maturational processes. Disorders of these suppressor and helper interactions have been defined in the primary immunodeficiency, autoimmune and malignancy diseases of man. Overall the studies are directed at defining disorders of the host immunoregulation that lead to high incidence of malignancy or that led to high incidence of infection in patients who have malignancies of the T and B cell system. Finally studies were directed toward developing new techniques for the detection of circulating tumor-related antigens that could be used in cancer diagnosis or in monitoring the effectiveness of therapy.

Methods Employed: New in vitro culture techniques of pokeweed mitogen stimulated peripheral blood lymphocytes have been developed for the study of the terminal differentiation of B lymphocytes into immunoglobulin secreting cells. New techniques with B cells purified by immunoabsorbent columns and followed by removal of contaminating T cells by rosetting techniques have been used in co-culture studies to detect helper T cells in man. Co-culture studies have also been utilized for the detection of excessive numbers or reduced numbers of suppressor T lymphocytes.

Major Findings: A major effort of the Metabolism Branch over the past few years has been directed toward defining the major events of cellular differentiation, cellular interaction and cellular biosynthesis involved in the specific circulating immune response. These studies have placed special emphasis on defining the defects of regulatory T cell and macrophage interaction with B lymphocytes and plasma cells that occur in patients with primary immunodeficiency diseases associated with a high incidence of malignancy, in patients with autoimmune disorders as well as in patients with malignancies of the T or B lymphocyte systems. Overall these studies were directed at defining the factors in normal and abnormal states controlling the production of antibodies and the synthesis of immunoglobulin molecules. Cells that ultimately produce antibodies undergo sequential maturation from stem cells in the marrow to B lymphocytes and then from B lymphocytes into immunoglobulin synthesizing plasma cells. The maturation of B cells into plasma cells is carefully regulated both positively and negatively by a series of separate cells. Specifically many antigens and mitogens require the presence of both helper lymphocytes of thymic origin (helper T cells) and macrophages as well as the B cells to induce a full antibody response. More recently it has been recognized that a separate class of thymic derived cells, suppressor T cells, may act as negative regulators of B cell maturation inhibiting this process. To study these events we have developed a series of techniques including techniques to study the terminal differentiation of B cells into immunoglobulin synthesizing and secreting cells, techniques to assess helper T cell function, to assess the macrophage contribution to these events and to detect both increased and decreased functional activities of suppressor T

cells. In the basic procedure peripheral blood lymphocytes are cultured with polyclonal activators of B cells in vitro and the immunoglobulin synthesized and secreted by these cells is quantitated using radioimmunoassay procedures. The first agent that we used in these procedures, pokeweed mitogen, is a prototype of a thymic and macrophage dependent activator of B cells. When pokeweed mitogen was added to unseparated mononuclear cells from the peripheral blood of normal individuals they stimulated B lymphocytes to mature into plasma cells which synthesize and secrete immunoglobulin molecules. However, when the B cells and macrophages were depleted of T cells they synthesized virtually no immunoglobulin molecules. When normal T cells were added to these B cells, they restored immunoglobulin synthesis to within the normal range. To determine if a population of cells had helper functional activity, they were co-cultured with the purified B cells and macrophages and the synthesis of immunoglobulins was detected. Pokeweed mitogen stimulation was also shown to be macrophage dependent. That is if B cells and T cells were exposed to pokeweed mitogen in the absence of macrophages, no immunoglobulin synthesis ensued. Indeed there was the generation of suppressor T cells. These studies support the view that macrophage presentation of antigens and mitogens to B cells is required for a normal immune response. Over the past year a new promising polyclonal activator of B cell maturation, an extract of Nocardia opaca has been utilized that appears to be less thymic dependent. Thus, we have developed both a mimic of thymic dependent antigens in pokeweed mitogen and of thymic independent antigens in Nocardia opaca. Finally, techniques for the study of deficiency and excesses of suppressor cell action have been developed.

Over the past year a series of studies were initiated to determine whether homogenous populations of T cells from patients with T cell leukemias retain the capacity to help or suppress the synthesis of immunoglobulins from lymphocytes derived from normal individuals. It has hoped that the study of the monoclonal proliferations of malignant T cells and their products may prove as exciting in answering questions regarding cellular immunity and T cell regulation of humoral immune responses as myeloma cells and their paraproteins have proved to be in answering questions concerning humoral immunity. The initial studies in this area focused on patients with the Sézary syndrome. This syndrome is a life-threatening disease characterized by exfolative erythroderma, generalized lymphodenopathy and circulating neoplastic T cells. Lymphocytes from patients with the Sézary syndrome and high numbers of circulating neoplastic T cells synthesizes essentially no immunoglobulin in pokeweed mitogen stimulated cultures in vitro. In addition, lymphocytes from the patients with the Sézary syndrome possessed no suppressor capacity. However, the circulating lymphocytes from approximately 70% of the patients with the Sézary syndrome studied functioned as helper T cells. There was a six to over 30-fold augmentation of immunoglobulin production by normal purified B cells after the addition of Sézary cells. These studies are in accord with our view that the Sézary syndrome in many cases represents a homogenous malignant expansion of helper T cells.

Patients with acute lymphocytic T cell leukemia present with a different pattern from those with the Sézary syndrome. The T cells from these patients with acute T cell leukemia did not function as helper cells in co-culture with normal B cells. However, in one of the four cases studied, the cells of the patient suppressed immunoglobulin synthesis by co-cultured normal cells in the presence of pokeweed mitogen. These cells were not cytotoxic to normal cells nor did they use up required media components by overgrowth of the normal cells. Thus, this patient appears to have a leukemia of the suppressor T cell population or of a population of T cells that can activate precursor cells from the co-cultured normal population to become suppressor T cells.

The majority of patients with the primary immunodeficiency states and immunoglobulin deficiency do not have a lack of helper T cells as their fundamental defect. However, we have demonstrated two children with thymic deficiency states that provide an interesting exception to this generalization. In these two children with associated severe thymic deficiency there was a markedly reduced immunoglobulin synthesis as well. When the lymphocytes were co-cultured with normal T cells or T cells from patients with the Sézary T cell leukemia, they synthesized large quantities of immunoglobulin molecules. Thus, in rare circumstances an isolated defect in helper T cells may lead to immunoglobulin and antibody deficiency even when a normal B cell-plasma cell system is present. For such patients appropriate therapy would be the transplantation of cultured epithelial thymus.

When the techniques of immunoglobulin biosynthesis were applied to the study of patients with common variable hypogammaglobulinemia, the patients were shown to be heterogenous with a variety of different immunological defects. The majority of the patients had a primary defect of the B cell plasma cell system. In some cases this was associated with the secondary development of suppressor T cells. In a small percentage of the patients there was a primary defect of suppressor cells that prevented B cells from maturing into plasma cells. Such a development was noted especially in individuals who developed immunodeficiency following infectious mononucleosis. The nature of the defect in patients with selective IgA deficiency was also investigated using in vitro techniques to study pokeweed mitogen driven terminal maturation of B lymphocytes into immunoglobulin synthesizing and secreting cells. Two patterns emerged from these patient studies. In the majority of the patients there was an intrinsic defect of the B cell-plasma cell series that normally developed into IgA producing cells. In a second group of patients, however, in addition to the defect in the B cells and plasma cells there was an increase in the number or state of activation of IgA class specific suppressor cells that prevent B cells from maturing into IgA synthesizing and secreting cells.

In the previous studies the disordered suppressor cells was of the T cell series. Patients with multiple myeloma appear to have a different defect as the cause of their significant reduction in serum levels of polyclonal immunoglobulins and in the capacity to synthesize antibody in response to antigenic challenge. These patients have markedly reduced polyclonal immunoglobulin

synthesis in vitro. In addition, in co-culture with normal cells they have a suppressor. However, in contrast to the observations in patients with common variable hypogammaglobulinemia or thymoma and hypogammaglobulinemia the cell appears to be a phagocytic mononuclear cell. Over the past year using an experimental model of transplantable myeloma in mice, we have demonstrated that the suppressor cell is a monocyte. Thus, both in the animal model and in man the hypogammaglobulinemia is due in part to the activation of immunoregulatory macrophage-like cells.

As discussed above excessive numbers of activated suppressor T cells may be associated with hypogammaglobulinemia and other forms of immunodeficiency. However, it should be noted that such cells normally play a key role in the negative control of the immune system in general and in the development of specific immunological tolerance. We have used an animal model system of autoimmunity, that of the NZB/W mice to analyze this question. These mice develop an autoimmune complex similar to that observed in patients with systemic lupus erythematosus. To approach the question of T cell suppressor function in NZB/W mice more directly we have taken advantage of the observation that splenic T cells can be activated by Concanavalin A to become cells which suppress the immunological function of other cells. Using this system we have shown that the NZB/W mice lose precursors of suppressor T cells as they age. In addition, they lose the capacity to secrete a normal physiological suppressor protein, soluble immune response suppressor. Although these animals cannot generate negative signals they can respond effectively to suppressor T cells generated from normal spleens and to the soluble immune response suppressor derived from normal T cells. When NZB/W mice were treated with SIRS from normal animals from an early age, they developed hypogammaglobulinemia as well as the abrogation of all manifestations of autoimmunity including the development of anti-DNA antibodies, Coomb's positive hemolytic anemia and immune complex glomerulonephritis. In addition, 90% of treated animals were alive after one year of treatment, whereas in the control group, only 10% of the animals were surviving at this time. Further studies are underway to find the active factor in the Concanavalin A supernatants, to determine whether established disease can be reversed by SIRS treatment and to determine whether patients with autoimmunity also lack suppressor T cell function.

The studies using sensitive radioimmunoassays for the oncofetal proteins alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) have continued. Over the past year a new assay for radioimmunoassay for alpha-fetoprotein has been established that significantly extends the lower limit of detectability of this protein. There are virtually no elevations of alpha-fetoprotein in benign conditions and physiological states outside of pregnancy, the disease ataxia telangiectasia and disorders associated with extreme hepatocellular regeneration. In extension of our previous studies we have found that 79 of 80 patients with ataxia telangiectasia have an elevated alpha-fetoprotein level whereas no other immunodeficiency or neurological state has such a defect. This retention of an oncofetal protein in this disorder supports the view that there is a failure of differentiation and

maturation for those systems that require the interaction of an entodermal analage with a mesenchymal inducer.

The combined markers alpha-fetoprotein and human chorionic gonadotropin are clearly of great value as markers for following the therapy of hepatocellular tumors, germ cell tumors of the testis other than semonomas. There have been no false positive values utilizing these markers and only 10% false negative values. It has clearly been shown over the past year that alpha-fetoprotein and human chorionic gonadotropin are produced by separate cells in the testicular tumors and that one may have a discordance of these markers during chemotherapy when there is a difference in the response of the different tumor elements to therapy. Thus, it is well established that both markers should be measured at the time of diagnosis and that both markers should be followed during patient therapy.

Significance to Biomedical Research: The techniques developed for the study of the terminal differentiation of B cells and for helper and suppressor T cells is of the same value in the study of B cell maturation as is the lymphocyte blastogenic response to PHA in the study of thymus-dependent lymphocyte (T cell) maturation. Thus, in vitro techniques are now available to evaluate both arms of specific immune response in man. One can now study the physiological factors regulating this response, one can define defects in the process in patients with immunodeficiency disorders or neoplasia of the B cell or T cell lymphocyte system. In addition, such studies can be used to determine the effect of chemotherapeutic or other agents of the terminal differentiation of B cells in parallel with studies of the effects of these agents on T cells so that one can define agents which preferentially affect T cell immunity and those which preferentially effect B cell immunity. Detection of circulating tumor-related antigens is of significance in the diagnosis of neoplastic disease and is of special relevance in evaluating the activity of the neoplastic disease and determining the efficacy of therapy of these disorders.

Proposed Course: Studies directed toward understanding the pathways and controlling mechanisms of the synthesis, transport and catabolism of serum physiological and pathophysiological factors controlling the sequential development of B cells into immunoglobulin synthesizing cells. Special emphasis will also be placed on the development of new techniques for the detection of circulating tumor-related antigens. These latter studies will include the evaluation of the synthetic rates of catabolism of such tumor-related antigens.

Honors and Awards

Bela Schick Memorial Lecture, 1977
Keynote Speaker, Alpha Omega Alpha Symposium, Duke University, 1977
Fifteenth Michael Heidelburger Award, 1976

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4003(c)-08-MET

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Porphyrin Metabolism in the Tumor-Bearing Host and Porphyria

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald P. Tschudy, M.D.	Senior Investigator	MET	NCI
OTHER:	Joel M. Lamon, M.D.	Clinical Associate	MET	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

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☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☑ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The main objectives are to study the mechanisms controlling heme biosynthesis and biochemical and clinical aspects of the porphyrias. To apply the knowledge gained from the above objectives to the study of tumors and tumor-host relationships as well as primary diseases of porphyrin metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To study the mechanisms controlling heme biosynthesis and biochemical and clinical aspects of the porphyrias. To apply the knowledge gained from the above objectives to the study of tumors and tumor-bearing relationships as well as primary diseases of porphyrin metabolism.

Methods Employed: Quantitative enzyme determinations, chemical determination of porphyrins and porphyrin precursors, isolation, purification and measurement of tissue heme, use of tissue preparations and isotope methods in studying metabolic pathways and computer and mathematical analysis of certain mathematical models.

Major Findings: 1. The effect of hematin on the biochemical and clinical manifestations of acute intermittent porphyria has been studied by administration of 19 courses of this agent in 11 patients. A course is defined as 3 mg/kg of hematin intravenously, daily for periods of 3-14 days. In all patients intravenous hematin caused a decline of urinary amino-levulinic acid (ALA) and porphobilinogen (PBG) excretion. Values of these porphyrin precursors can be decreased to normal levels by continuing therapy at doses which have no side effects and no evidence of toxicity. The overall clinical impression thus far is that hematin appears to be of value in ending acute manifestations of the disease. The conclusion has also been reached by patients, their relatives and certain outside physicians who have collab-rated on this study. As might be expected, however, established motor neuro-pathy is not dramatically effected. In one patient with cyclic attacks, administration of hematin once a week may be preventing the recurrence of these attacks.

2. Hemopexin levels in blood have been shown to be increased in patients with acute intermittent porphyria and to increase further during acute attacks of the disease. Hematin therapy lowers blood hemopexin to unmeasurable levels.

3. Intravenously administered hematin has been shown to function as the prosthetic group of tryptophan pyrrolase in liver in addition to its capacity to act in the repression of hepatic ALA synthetase.

4. Hyperamylasemia has been shown to be associated with acute inter-mittent porphyria in four patients. In one patient this was macroamylase.

5. Chemical manifestations of hyperthyroidism have been observed in 10 patients with acute intermittent porphyria, two of whom have been treated for flinically evident hyperthyroidism.

6. Another red cell enzyme test for acute intermittent porphyria has been developed. It measures uroporphyrinogen I synthetase activity by a spectrophotometric rather than fluorimetric method and utilized the cheaper and more readily available δ-aminolevulinic acid rather than porphobilinogen as substrate. It is thus easier for clinical labs to perform than the previously described fluorimetric method.

7. Family studies of the correlation of the red cell enzyme defect of acute intermittent porphyria and the abnormal chemical findings in the urine show that a significant number of afflicted individuals cannot be detected by urine testing.

8. Studies of HLA antigens have shown no correlation with the various hepatic porphyrias.

9. The kinetics of the heme biosynthetic pathway in liver is being studied in detail by use of isotopic δ-aminolevulinic acid.

Significance to Biomedical Research: Mechanisms of the control of metabolic pathways and the synthesis of enzymes in normal and diseased tissues are being elucidated. The findings have been directed at both theoretical and practical goals. In the former realm, models are being developed to explain the behavior of the pathway and its control system under normal and pathological conditions. In the practical realm, methods of diagnosis and treatment of the porphyrias are being evolved. The abnormalities of the control of heme biosynthesis in tumors and host livers which have previously been demonstrated in this laboratory will be further studied by the methods developed for experimental and human porphyria.

Proposed Course: (A) To continue studies of the factors controlling heme and heme-enzyme synthesis in normal and pathologic tissues. (B) To continue studies of biochemical abnormalities of porphyria in man and experimental animals. (C) To study control mechanisms of heme synthesis in tumors and livers of tumor-bearing hosts.

Publications:

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Lamon, J.M. and Tschudy, D.P.: The porphyrias. Current Therapy, 1979. In press.

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Tschudy, D.P.: The influence of hormonal and nutritional factors on the effect of chemicals on liver haem and haemoprotein metatolism. In DeMatteis, F. and Aldridge, W.N. (eds.): Effect of Chemicals on Haem and Haemoprotein Metabolism. Springer-Verlag. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4004(c)-07-MET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Amino Acid Biosynthesis and Degradation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James M. Phang, M.D.	Senior Investigator	MET	NCI
OTHER:	Robert J. Smith, M.D.	Clinical Associate	MET	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Metabolism Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	3	4

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☑ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An important factor in cellular growth and metabolism is the biosynthesis degradation, and transport of amino acids. This project emphasizes the regulatory interplay in amino acid metabolism.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The biosynthesis, degradation and transport of amino acids play an important role in cellular growth and metabolism. The mechanisms by which these processes are regulated in normal and malignant cells are poorly understood. The project emphasizes the regulatory interplay in amino acid metabolism. It is relevant to Objective 3, Approaches 1 and 5 of the National Cancer Program.

Methods Employed: We are primarily using animal cells maintained in long-term tissue culture. Cells can be cloned to insure a genetically homogeneous population. Mutants are isolated by standard techniques. A variety of biochemical techniques have been utilized to study amino acid biosynthesis and degradation in cultured cells. Uptake of amino acids may be assessed by using labeled L-amino acids and rates of protein and collagen synthesis can be obtained by incubating cells with L-proline-C^{14} and separation of the radioisotopically incorporated amino acids as proline and hydroxyproline by ion exchange column chromatography. The enzymes for biosynthesis and degradation of proline are assayed by using appropriate labeled precursors and isolating the products by ion exchange chromatography. Our methods are sensitive enough to measure these enzymes in leucocytes isolated from 10 ml of blood.

Major Findings: In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminates ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps reamin undefined in mammalina cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase. Hydroxyproline degradation follows a scheme analogous to that for proline. Hydroxyproline is converted first to 3-OH-PC and then to γ-OH glutamate.

Proline oxidase, the enzyme which catalyzes the first step in proline degradation, has been emphasized during the past year. The enzyme is tightly bound to mitochondrial membranes and the protons generated by proline oxidation are directly transferred to cytochrome C of the electron transport chain. In addition, the carbons from proline degradation directly contributes to the TCA cycle and carbohydrates. Due to these considerations, proline oxidase and its regulation is of major importance.

We first demonstrated that proline oxidase is localized to certain tissues. In the order of decreasing specific activities, liver, heart, kidney and intestinal mucosa have proline oxidase. In contrast a variety of other tissues have no detectable proline oxidase. These include: skeletal muscle, circulating blood cells, cartilage (epiphyseal, xiphoid, and costochondral) membranous and diaphyseal bone and skin.

Glucose production from proline was directly shown using *in vitro* incubations of rat liver minces. We determined the production of both total

glucose and glucose-^{14}C from precursor proline-^{14}C. After an initial 10 minute delay, accumulation of glucose in the media was linear for 60 minutes. With increasing media proline concentration glucose accumulation increased but plateaued with proline at 5 mM. Relative to glucose production the half-maximal concentration for proline was about 2.5 mM, a concentration similar to the Km of proline oxidase for proline. At saturating concentrations of amino acid precursors, glucose production from proline was about 50% of that from alanine, a well-known amino acid source for gluconeogenesis. These studies clearly show that livers can utilize proline as a significant source for gluconeogenesis.

Since mammalian organisms have a number of sources for metabolic fuel, it would be advantageous to regulate amino acid degradation when alternative fuel sources are available. With high rates of glycolysis (e.g., exercise) lactate formed and released by working muscle is taken up by liver and converted back into glucose. We found that lactate reversibly and specifically inhibits proline oxidase thus linking the rate of proline degradation to carbohydrate metabolism. Inhibition of proline oxidase increases with increasing lactate concentration. With proline concentration at 0.1 mM, lactate at 5 mM inhibits 90% of the enzyme activity. The lactate effect is specific to proline oxidase. Lactate at 5 mM was without effect on a number of other cytosol and mitochondrial enzymes. The lactate effect was also specific in that inhibition was not generalizable to other metabolic intermediates. Besides lactate, only pyruvate and succinate inhibited proline oxidase. The modest inhibition by pyruvate and succinate, however, was seen only at supraphysiologic concentrations. A variety of other intermediates (alpha-ketoglutarate, glucose, glutamate, fumarate, oxalacetate, acetoacetate, acetyl-CoA, B-hydroxy butyrate, alpha-ketoisocaproate) were without effect. Inhibition of proline oxidase by lactate was completely reversible. After exposure to lactate at 37° for 30 minutes, mitochondria recovered by washing and centrifugation had full enzyme activity. More importantly, enzyme solubilized using Trition X-100 was inhibited by lactate thus ruling out interference by lactate of proline entry into mitochondria as the basis for the inhibitory effect. Kinetic studies showed that lactate increased the Km of proline oxidase for substrate proline but was without effect on enzyme Vmax. Kinetic studies with lactate at a variety of concentrations showed that the Km of proline oxidase for proline increased linearly as a function of lactate concentration.

These in vitro observations were corroborated by previously published observations in man where hyperprolinemia has been associated with genetic and acquired lactic acidosis. Our in vitro observations may be the basis for this previously unexplained metabolic derangement. Furthermore the regulation of proline oxidation by lactate may serve as a model for control of gluconeogenesis and metabolic fuel allocation.

Significance to Biomedical Research: The regulation of amino acids for protein synthesis may play an important role in controlling cell growth. The enzymes involved in amino acid biosynthesis and degradation are affected by genetic, nutritional and hormonal factors. Our studies on the proline metabolic enzymes are contributing to the understanding of these factors. Speci-

fically, genetic defects in the proline metabolic enzymes have elucidated some of the physiologic derangements secondary to a metabolic block. Elucidation of the molecular mechanisms for regulating proline biosynthesis and degradation will lead to an understanding of biochemical regulation in mammalian cells.

Proposed Course: Our recent finding that proline oxidase is regulated by lactate together with our previous findings of developmental and hormonal alterations in enzyme activity emphasize the importance of proline degradation. The regulation by lactate suggests that other metabolic intermediates also may be effectors of enzyme activity. Initial observations show that long chain fatty acid acyl coenzyme A's may regulate enzyme activity. These regulatory effects suggest that the enzyme may have binding sites for these effectors. Furthermore, modulation of these effector sites may exert additional regulation of enzyme activity. We will seek modulation of these sites by hormones, e.g., cyclic adenosine monophosphate. Phosphorylation of adenylation may be involved in this modulation. Another major area of interest will be the physiologic significance of proline oxidation. We already have shown that glucose may be produced from proline. We will quantitate this contribution and compare the magnitude of this contribution to that made by alanine. Furthermore, we are seeking to define the anaplerotic role to proline oxidation. Altered mitochondrial function concommitant with proline oxidation also may be a possibility. PRO/RE mice, an inbred strain with proline oxidase deficiency, will provide some experimental approaches. Finally, we are attempting to solubilize and purify proline oxidase from rat liver mitochondria. Studies on the regulation of the purified protein will allow for definitive demonstration of the mechanism of enzyme regulation.

Publications:

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Kowaloff, E.M., Granger, A.S. and Phang, J.M.: Alterations in proline metabolic enzymes with mammalian development. Metabolism 25:1087-1094, 1976.

Kowaloff, E.M., Granger, A.S. and Phang, J.M.: Glucocorticoid control of hepatic proline oxidase. Metabolism. In press.

Downing, S.J., Phang, J.M., Kowaloff, E.M. and Smith, R.J.: Proline oxidase in cultured mammalian cells. J. Cell. Physiology. In press.

Smith, R.J., Downing, S.J. and Phang, J.M.: Synthesis and purification of L-pyrroline-5-carboxylic acid. Anal. Biochem. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4014(c)-08-MET

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of the Immune Response in Normal and Pathological States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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Metabolism Branch

SECTION

Immunophysiology Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	0

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☑ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were directed toward the analysis of immune response in normal humans and patients with a variety of immune disorders, as well as analysis of humoral and cellular immune responses of experimental animals.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Studies were directed toward the analysis of humoral and cellular immune responses of experimental animals with particular emphasis on the study of experimental models of human disease. Studies were directed toward the analysis of the human response in normal humans and patients with a variety of immune disorders. This included the study of patients with gluten-sensitive enteropathy, dermatitis herpetiformis, inflammatory bowel disease, as well as patients with a variety of immune deficiency diseases, such as ataxia telangiectasia, common variable hypogammaglobulinemia and intestinal lymphangiectasia. These diseases are each associated with a high incidence of reticuloendothelial malignancy and the mechanism underlying this association was sought.

Methods Employed: A host of techniques were used to assess immunologic function of experimental animals and in patients with disorders of the immune system. Circulating B-cells in peripheral blood and tissue were enumerated with carefully prepared fluorescein-conjugated antiserums with Ig specificity. Circulating T-cells were enumerated by sheep red blood cell rosette techniques. Antibody responses were assessed with microhemagglutinination techniques and in vitro proliferative responses were assessed with tritiated thymidine incorporation following exposure to specific and nonspecific mitogens. The presence of mitogen induced cytotoxic effector cells were assessed with a ^{51}chromium release assay, using red cell and nucleated, metabolically active cell targets. The presence of antibody-mediated cytotoxic effector cells was assessed with ^{51}chromium release assays using red cell and nucleated cell targets in the presence of appropriate antibodies. Cells were isolated with the use of Sephadex anti-Fab columns, rosetting techniques, use of colloidal iron followed by passage through a magnetic field and differential binding to antigen-antibody plates. Short term (1-2 hour) organ culture was used in conjunction with a solid phase immunoabsorption technique to measure local mucosal immunoglobulin synthesis. Long term (24-48 hour) organ culture was used to study in vitro models of intestinal disease. Radioimmunoassays in conjunction with lymphocyte culture in the presence of mitogen was used to measure terminal B-cell differentiation. Patients with immunologic disorders were studied with immunoelectrophoresis, radial immunodiffusion and metabolic turnover techniques for measurement of immunoglobulin synthesis and catabolism.

Major Findings: Gluten-sensitive enteropathy. Studies directed toward understanding the pathogenesis and treatment of gluten-sensitive enteropathy (GSE) were continued. GSE is a disease of the GI tract associated with malabsorption, villous flattening, inflammatory cell infiltration of the GI tract and an increased incidence of neoplasia. It is due to the toxic effect of wheat proteins (gluten) on the gastrointestinal mucosa. In previous studies we observed that approximately 80% of the patients with GSE bear the HLA-B8 histocompatibility antigen. A similar incidence of this antigen was observed in dermatitis herpetiformis, a skin disease associated with gastrointestinal lesion essentially identical to that found in GSE. In current studies we have determined that GSE is associated with a high incidence of a

second histocompatibility antigen, HLA-DW3. This antigen is detected with appropriately absorbed antisera used in conjunction with a cytotoxicity assay in which the target cells are peripheral blood B-cells isolated with the use of anti-FAB coated microtiter plates. We have found that approximately 80% of GSE patients bear HLA-DW3 in contrast to 35% of random control individuals. HLA-DW3-positivity was found among both HLA-B8 positive and HLA-B8 negative individuals.

At this point in our knowledge it is not clear whether the HLA genes responsible for the antigens associated with GSE take a direct part in the disease mechanism underlying GSE or, alternatively, are linked to an as yet unidentified gene, which is the true "GSE gene." Evidence for the former possibility was obtained during this period in a study done in collaboration with Dr. Z.M. Falchuk of the Peter Bent Brigham Hospital, we found that patient responses in in vitro organ culture depended on HLA status. More specifically in a prospective study of patients with diarrheal illness, tissue obtained from 23 GSE patients were subjected to organ culture in the presence and absence of gluten-protein (peptic-tryptic digest of culture). Nineteen of the 23 patients demonstrated gluten toxicity in organ culture: failure to undergo epithelial cell maturation; all 19 of these patients were HLA-B8 positive. The four remaining patients failed to demonstrate gluten toxicity in organ culture; three out of four of the patients were HLA-B8 negative. In companion studies using purified gliadin in the organ culture system it was observed that 10 of 11 HLA-B8 positive patients demonstrated gluten toxicity in organ culture whereas seven of eight HLA-B8 negative patients failed to demonstrate gluten toxicity in organ culture. These studies point to the conclusion that "HLA-B8 status" in some measure determines the degree of toxicity to gluten protein. This is likely to be due to the fact that the cell surface configuration which recognizes gluten on the epithelial cell is made up, at least in part, by the histocompatibility antigen.

It has been clear, as a result of analysis of the inheritance of GSE in families studied in relation to the inheritance of HLA antigens, that HLA antigens cannot be the only genetic determinants important to the occurrence of GSE. In this regard, we have recently discovered a second genetically-determined cell surface protein that is associated with GSE, the GSE-associated B-cell antigen. This specificity is found on peripheral B-cells (but not T-cells) of patients and is detected with antisera from mothers of patients who were presumably immunized with pateint cells during pregnancy. Using a cytotoxicity test specific for B-cells we determined that the B-cell antigen is present in 90% of patients, but only 20% of normals. Since the sources of the antisera, the mothers of patients, were themselves HLA-DW3 positive and, further since some patients and controls were discordant for GSE-associated B-cell antigens and HLA-DW3 it is likely that the GSE-associated B-cell antigens and HLA-DW3 represent separate specificities.

Using the typing methods for HLA-DW3 and GSE-associated B-cell antigens alluded to above, individuals in seven families of patients with GSE were characterized by antigen type. As a result, several conclusions concerning

the genetic background of GSE can now be drawn: 1) It was found that all parents of patients were positive; since only 20% of random individuals are positive the high degree of positivity among parents strongly suggests that a pre-condition for the occurrence of GSE is the inheritance of the inheritance of the appropriate B-cell antigen from both parents; in other works, patients are homozygous for the GSE-associated B-cell antigen; 2) most patients were positive for HLA-DW3 as well as the GSE-associated B-cell antigen; it is, therefore, likely that at least a single dose of the gene controlling HLA-DW3 (or a related specificity) is necessary for the occurrence of GSE; 3) the inheritance of the GSE-associated B-cell antigen and HLA antigen was discordant, that is in several families siblings were found who were GSE-associated B-cell antigen identical, but HLA non-identical. These studies, in summary, indicate that GSE is controlled, at least in part, by two non-linked genes, one in the major histocompatibility locus and one on a separate chromosome not containing the major histocompatibility locus.

The foregoing genetic study should not be construed as indicating that the genes identified are the only genetic or environmental antecedents of GSE. In this period we have studied a set of twins, one with GSE and one without GSE. This twin-pair were proven identical on the basis of at least six different polymorphic systems including red cell isoenzymes and surface antigens, B-cell antigens, HLA antigens and fingerprint studies. The discordancy in this identical twin pair gives rise to the expectation that environmental factors will ultimately be found to influence the occurrence of GSE.

During this period we have initiated studies on the nature of the association between B-cell antigens and GSE. In this regard, an important observation has been that the maternal antiserums recognizing the GSW-associated B-cell antigen fails to induce lymphocyte cytotoxicity when cells from patients are pre-incubated with gluten-proteins. This observation implies that the GSE-associated B-cell antigen is a receptor molecule for gluten protein and as such, is important in the initiation of the immune responses which are central to the pathogenetic mechanism underlying GSE.

In other studies we have begun a formal therapeutic trial of a bread prepared from a genetic strain of wheat which lacks α-gliadin. This wheat was grown up, milled and made into bread in conjunction with Dr. D. Kasarda of the U.S. Agricultural Department. The bread is currently being fed to patients under controlled conditions and so far appears to be far less toxic to patients than ordinary bread.

Studies of the regulation of gastrointestinal immune responses. During this period a broad study of the regulation of gastrointestinal immune responses was initiated. Initial attention was directed toward the culture of organized gut-associated lymphoid tissue (Peyer's patch lymphocytes). It was found that Peyer's patch lymphocytes responded poorly in culture with respect to either proliferation (^{3}H-thymidine incorporation) or immunoglobulin production unless 2-mercaptoethanol was added to the culture at an appropriate concentration. In an analysis of this phenomenon it was found that a sonicate

of intestinal epithelial cells was totally suppressive to proliferation of cells obtained from a variety of organs. Since this suppression could be completely abrogated by addition of 2-mercaptoethanol, it was reasoned that a suppressor substance derived from epithelial cells accounts for the unreactivity of Peyer's patch cells. Moreover, since purified Peyer's patch lymphocytes are unresponsive in culture even when separated from other cellular elements, the suppressive factor appears to exert its effect while the cell is still in the micro-environment of the Peyer's patch.

In related studies, a means of measuring mouse IgA biosynthesis were devised. This consisted of culturing washed cells in vitro in the presence of various mitogens, including the B-cell mitogen, lipopolysaccharide and then measuring IgA biosynthesis over a seven day period with an IgA-specific double radioimmunoassay. With this technique substantial IgA synthesis in vitro has been observed and the way is clear to use this test system in the study of the regulation of IgA response in vitro.

Significance to Biomedical Research: In the studies conducted during the past year we have identified a surface antigen which may be a receptor protein for the toxic agent specific to this disease, gluten protein. This discovery will allow us to analyze the relation between histocompatibility antigens and gluten-sensitive enteropathy since it is possible that the receptor protein identified above is physically related to surface proteins coded for by histocompatibility antigens. By the same token, it will be possible to analyze the role of histocompatibility antigens in the genesis of deleterious immune responses; such information will derive from the antigenic stimulation of GSE lymphocytes in vitro and the modification of such responses by antisera recognizing histocompatibility antigens and other cell surface antigens.

Studies of the regulation of the gastrointestinal immune response are important to the understanding of major gastrointestinal diseases, all of which are associated with an increased risk of cancer. In this regard, the inflammatory bowel diseases may be thought of as disorders of immuen regulation in which inappropriate responses to normal gut constituents form the basis of the pathologic process. A more profound understanding of the factors controlling GI immune responses may therefore provide insight into the cause of these inflammatory states.

Proposed Course: Studies on the nature of gluten-sensitive enteropathy will be continued. We have recently successfully placed into long term culture cells of two patients with GSE. These cultured cells have been shown to bear GSE-associated B-cell antigens. We propose to fractionate the cell membranes of these cultured cells using KCL or deosycholate extraction techniques. We then propose to raise heterologous antisera against purified membrane components in order to obtain stronger, more specific antisera with specificity for GSE-associated antigens. With the help of such antisera we propose to study the relation between GSE-associated B-cell antigens and HLA antigens on the cell membrane. More specifically, we will use immunoprecipitation and polyacrylamide gel techniques to determine if the GSE-associated B-cell antigen and the HLA antigen are physically associated on the cell membrane.

In other studies we will use the maternal antisera as well as heterologous antisera described above to study the relationship between the GSE-associated B cell antigen and the initiation of gliadin specific immunostimulation in vitro. In these studies we will stimulate lymphokine production (leukocyte inhibitory factor, LIF) by patient cells in vitro with gliadin protein. We will then attempt to inhibit such LIF production by pre-incubation of cells with purified IgG fractions of anti-GSE-associated B-cell antigen or by preincubation of cells and exposure to complement. In this way, we hope to show that relevant B-cell antigens are important in the initiation of immune processes subtended by gliadin.

In yet other studies we propose to demonstrate binding of purified gliadin factions to both lymphoid cells and gut epithelial cells. In this connection we also hope to demonstrate gliadin binding to skin of DH patients since it is likely that such binding accounts for the skin lesions encountered in such disease. Purified gliadin components will be made available through cooperating units in the Department of Agriculture and will be used in organ culture studies to define more precisely the nature of the toxic chemical configuration important to GSE.

In studies of the immunology of the gastrointestinal tract we propose to analyze to define the enhancing and suppressing activity of Peyer's patch T-cells as well as epithelial cells and epithelial cell products. In these studies we plan to culture purified T-cells obtained from various organ sources as well as subpopulations of T-cells obtained from these sources with purified B-cells from either Peyer's patches, spleen or mesenteric nodes. Following culture we plan to analyze biosynthetic activity of the cultured B-cells by performing radioimmunoassay on culture supernatants. In this way we intend to define the regulatory factors operating in IgA biosynthesis.

Publications:

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Peña, A.S., Mann, D.L., Hague, N.E., Heck, J.A., and Strober, W.: The Genetic Basis of Gluten-Sensitive Enteropathy. Gastroenterology. In press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CB-4015(c)-08

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Development and Function of Humoral and Cellular Immune Mechanisms of Host Defense

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5 1/2	3	2 1/2

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is directed toward determining mechanisms important to the development and function of humoral and cellular immune responses; the nature of deficiency in immune function; and developing approaches to prophylaxis and/or therapy of infections and neoplastic disease associated with immune processes.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of this study were to determine mechanisms important to the development and function of both humoral and cellular immune response in animals and man; to determine the nature of the deficiency in immune function characterizing such disease states as hypogammaglobulinemia, ataxia telangiectasia, the Wiskott-Aldrich syndrome, intestinal lymphangiectasia and advanced malignancy; and, to develop approaches to prophylaxis and/or therapy of the infectious and neoplastic disease frequently associated with defective immune processes. This work is directly related to Objective 2, Approach 1 of the National Cancer Plan.

Methods Employed: Antibody responses of experimental animals and man were determined after immunization with bacterial vaccines such as brucella abortus, S. typhosa, and pertussis; with particulate cellular antigens such as sheep, burro, and chicken RBC's; with purified protein antigens such as KLH, diphtheria and tetanus toxoids, guinea pig-γ-globulin and bovine γ-globulin; with purified polysaccharide antigens such as pneumococcal polysaccharides types I, II, III, and the V_i antigen of E. coli. The antibodies were detected by standard techniques or by newly developed micro methods of bacterial agglutination, red cell hemolysis, and passive hemagglutination using $CrCl_3$ as an agent to couple the antigens to a carrier RBC surface. In addition, modifications of the Farr antigen binding assay were used.

Cellular immune responses were measured by delayed hypersensitivity skin tests, contact sensitivity to DNCB, and skin allografts. Lymphocyte transformation in vitro was carried out utilizing non-specific mitogens such as PHA, PWM and staph filtrate; specific antigens such as SLO, SKSD, diphtheria and tetanus toxoids, KLH and candida albicans; and allogeneic cells in mixed leukocyte culture. Lymphocyte function in vitro was also measured by production of mediators of cellular immunity such as lymphotoxin, MIF, and lymphocyte derived chemotactic factor for monocytes (LDCF) and by cytotoxicity reactions with antibody sensitized target cells or with non-specific mitogen activated effector lymphocytes.

Studies of the characteristics of various cell surface receptors on immunocompetent lymphocytes and monocytes-macrophages utilized auto-radiography, immunofluorescence microscopy, and cellular rosette formation.

Major Findings: Studies of the immunoregulatory role of T-cells have been continued in an animal model of humoral immunodeficiency. In the chicken, a central lymphoid organ, the Bursa of Fabricus is the site of initial differentiation and development of B lymphocytes. If the Bursa is removed in the newly hatched chicken before B cells have emigrated to peripheral lymphoid organs, the chicken develops agammaglobulinemia. This immune deficiency state is characterized by total absence of B lymphocytes, immunoglobulins in the serum and secretions are undetectable, and all lymphoid tissues lack their characteristic components associated with the system of humoral immunity. T lymphocyte differentiation and function is not deficient in these bursectomized agammaglobulinemic chickens. However T cells

obtained from these birds were found to exert a profound effect on the humoral immune system when they were transplanted into normal birds. Recipients of T cell grafts from agammaglobulinemic donors were found to have impaired antibody responses and to frequently develop agammaglobulinemia themselves. This transplantable or "infectious agammaglobulinemia" was found to be caused by suppressor T cells present in the transplanted cells from the original bursectomized donor. The immunodeficiency which developed in the recipient chickens was characterized by total absence of IgG, IgA, and IgM just as in the bursectomized donors, but differed from donor birds in that the recipient chickens had the persistence of B lymphocytes and germinal centers in their lymphoid tissues and continued to have a Bursa of Fabricus. Thus, these recipient chickens had developed an immunodeficiency state closely paralleling that seen in many patients with common variable hypogammaglobulinemia who also have persistence of B lymphocytes and evidence of excessive "suppressor T-cell" activity.

Further study of these birds with "infectious agammaglobulinemia" demonstrated that the suppressor T cells develop in the donor birds after 10 weeks of age and are not present in detectable quantities before that time. Immunoglobulin production in the recipient birds ceased almost immediately after injection with the suppressor cell population suggesting that these cells may be exerting an effect directly on the immunoglobulin producing plasma cells. B lymphocytes are initially present in normal numbers in the recipient birds but eventually fall to low numbers further suggesting that the suppressor cell may also act directly on B cells or their precursors.

Dysgammaglobulinemia in man is a disorder characterized by the deficiency of one or more classes of immunoglobulin with persistence of normal levels of the other immunoglobulins. When T cells from various agammaglobulinemic chickens were transplanted to normal recipients in limiting dilution assays, recipient birds were found to develop dysgammaglobulinemia with selective loss of only one class of immunoglobulin. The deficiency which ultimately developed in the recipient birds varied from donor to donor suggesting that suppressor cells for each individual class of immunoglobulin are present in the donors and that the predominant suppressor cell class is revealed by the limiting dilution assay. Antibody responses in the dysgammaglobulinemic birds were present in the non-suppressed Ig classes and as in the birds which developed agammaglobulinemia following transplantation, B cells continued to be present bearing the immunoglobulin of the suppressed Ig class. This Ig class specific suppression also closely parallels immune deficiency disorders seen in man and provides an excellent experimental model to further investigate the role of T cell regulation of Ig production and may allow the development of therapeutic approaches for the correction of such deficiencies in man.

Certain abnormal immunoregulatory mechanisms may result in immune deficiency. Similarly, other abnormalities in the regulation of immune responses may result in autoimmunity. A striking example of autoreactivity present in normal animals and man is the capacity of T lymphocytes to respond to B lymphocytes from the same individual. We have found that in vitro, this response is demonstrated as an autologous mixed lymphocyte reaction when T lymphocytes

are induced to undergo vigorous proliferation when exposed to autologous B lymphocytes. A variety of substances with immunoregulatory properties were studied for their effects on this form of lymphocyte autoreactivity. At physiological and supraphysiological concentrations of alpha-fetoprotein and the thymic hormone thymosin, no detectable effect was observed on the autologous MLR. Cortisol at pharmacologic concentrations has been shown to be suppressive to a variety of in vivo and in vitro lymphocyte functions. We found that at such concentrations in vitro, hydrocortisone had moderate suppressive effects on the allogeneic MLR and surprisingly totally abolished all autoreactivity as detected in the autologous MLR between T and B lymphocytes. This differential sensitivity of autoreactive T cells was found to be maintained even at physiologic concentrations of cortisol. Under these conditions, reactivity to allogeneic cells was completely unaffected while autoreactivity was inhibited by 98% or greater. Thus, concentrations of cortisol found normally in human plasma have a profound immunoregulatory role in selectively blocking autoreactivity of at least one class of immunocompetent T cells.

Studies of the mechanism of action of various cytotoxic cells and their contribution to host defense were continued. Model systems have been developed for the study of killer cells in two separate and distinct cytotoxic processes. Antibody dependent cellular cytotoxicity (ADCC) is a form of cell mediated killing in which non-immune effector cells attack and kill target cells sensitized with IgG antibody. Mitogen induced cellular cytotoxicity (MICC) is a form of cell mediated killing in which non-immune effector cells kill target cells in the presence of a mitogen or lectin. Both MICC and ADCC are surface mediated killing events which do not require phagocytosis of the target cell. The mechanism of the surface mediated cytotoxicity has been obscure. Inhibitors of effector cell DNA, RNA and protein synthesis have not in general inhibited the cytotoxic capacity of these cells. Quabain, a cardiac glycoside, functions as an inhibitor of Na, K and Ca^{++} membrane transport systems. Addition of quabain to MICC and ADCC reactions inhibited MICC by greater than 95% while inhibiting ADCC by approximately 50%. EDTA and EGTA, also inhibited MICC reactions to a significantly greater extent than ADCC. The inhibitory effects of these agents on MICC reactions could be completely reversed by the addition of Ca^{++} to the cell cultures. These data suggest a critical role for Ca^{++} ion flux in the cytotoxic action of certain effector cell populations and helps our understanding of the basic mechanisms by which effector cells mediate their killer potential.

Studies of the influence of macrophage function on the development and expression of immunocompetence in neonatal life were continued. Newborn rats are immunologically incompetent as determined by their failure to produce antibody when immunized with several antigens such as SRBC or KLH. They are also highly susceptible to lethal infections with the facultative intracellular organism listeria monocytogenes. A third aspect of their immunodeficiency is their high degree of susceptibility to the induction of specific immunological tolerance with high doses of antigen. Each of these parameters of immune incompetence can be corrected in the neonate by providing them with macrophages from adult donors or by treating the neonate with agents which activate their

own immature macrophage population. Studies of the nature of the macrophage requirement for the resistance to the induction of immunological tolerance revealed a close association between mature macrophage function and the generation of helper T cells upon exposure to antigen. By contrast, in the absence of mature macrophages (as in the normal newborn) exposure of T cells to antigen results in the preferential activation of suppressor T cells. These observations help to explain the susceptibility to tolerance induction in the neonate and also help to define another role for the macrophage in the interaction of immunocompetent cells.

Evaluation of the immune capacity of patients with the rare syndrome of familial lymphohistiocytosis were performed in collaboration with Drs. Ladisch and Poplack of P.O.B., NCI. This disorder is characterized by infiltration of lymphoid tissues with masses of normal appearing histiocytes and is associated with hepatosplenomegaly, erythrophagocytosis, hemolysis and consumption coagulopathy, recurrent fevers, wasting and increased susceptibility to infections. Immunologically the patients were found to be anergic to all recall skin test antigens. In vitro evidence of abnormal cellular immune function included depressed lymphocyte proliferative responses to specific antigens and impaired monocyte mediated cytotoxic function. Plasma from these patients suppressed the responses of normal lymphocytes. Investigation of the suppressive plasma revealed hyperlipidemia with triglyceride levels between 180 - 960 mg % (normal upper limit 150 mg %) and the degree of in vitro immunosuppression correlated with the extent of the hyperlipidemia. Further investigation of the immunosuppressive effects of plasma with hyperlipidemia showed that patients with a variety of disorders of lipid metabolism have in vitro and occasionally in vivo evidence of defective cellular immune function. Preliminary studies suggest that the immunosuppressive material in the plasma is associated with LDL and VLDL and may be GM ganglioside. This is a particularly intriguing observation since studies in mice have demonstrated that certain T cell associated antigens may bind to GM ganglioside and that this results in the activation of suppressor T cells.

Clinical studies of cytotoxic effector cell activity in various patient groups were continued. The antibody dependent cellular cytotoxicity (ADCC) reaction is dependent on the source of the antibody, the type of effector cell and the nature of the target cell. A special type of ADCC reaction utilizing human group B red blood cells as targets and human hyperimmune anti-B antibody was found to utilize monocytes and polymorphonuclear leukocytes as effector cells rather than lymphocytes as the killer cell. Utilizing this reaction as a clinical test of monocyte function we have observed several diseases with defective monocyte cytotoxic activity. The Wiskott-Aldrich syndrome is an x-linked recessive disease characterized by thrombocytopenia, eczema, and recurrent infections with all classes of microorganisms. Our studies have previously defined many defects in humoral and cellular immunity in boys with this disease. Although ADCC activity of the lymphocytes from these patients is normal, we found that monocyte mediated ADCC activity was virtually absent in these children. This abnormality appears to be an intrinsic defect in the cellular cytotoxic mechanism of these cells since they were found to have normal cell surface receptors capable of binding IgG coated erythrocytes and

even were able to phagocytize these target cells normally. Similar studies have demonstrated monocyte cytotoxic defects in patients with chronic mucocutaneous candidiasis, lymphohistocytosis and various childhood malignancies. Since the monocyte and macrophage plays a central role in many cellular immune reactions such as delayed hypersensitivity reactions and tumor rejection, such a defect in monocyte cytotoxic capacity may contribute a significant element of the cellular immune deficiency in these patients.

Significance to Biomedical Research: The present studies extend our understanding of the diverse, yet interrelated mechanisms contributing to the development and normal functioning of immune system in animals and man. They indicate that disorders of immune function may be related to the absence or malfunction of such central lymphoid organs as the thymus or Bursa of Fabricius or to immaturity of such auxillary systems as the macrophage required for the processing of antigens in the initiation of an immune response. In addition, they indicate that immunoregulatory cells and humoral materials may have a profound effect on the function of the immune system both in health and disease. These studies have also provided new insights into some basic defects in accessory immune elements which provide new avenues for work designed to correct malfunctions in immunity in malignant and immunodeficiency disorders.

Proposed Course: Continuing studies directed toward understanding the normal pathways involved in the development and function of the immune system and application of this new knowledge in approaches to the prevention and therapy of malignant disease.

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leukocyte (MNL) function. In Proceedings of the Eleventh Leukocyte Culture Conference, Lucas, D., ed. Academic Press (in press).

Gershwin, M.E., Blaese, R.M., Steinberg, A.D., Wistar, R. and Strober, W.: Antibodies to nucleic acids in congenital immune deficiency states, J. Pediatrics 89:377-381, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4016(c)-02-MET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanism of the Anabolic Action of Growth Hormone

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Peter Nissley, M.D.	Senior Investigator	MET	NCI
OTHER:	Alan C. Moses, M.D.	Clinical Associate	MET	NCI
	Gerhard Baumann, M.D.	Visiting Scientist	MET	NCI

COOPERATING UNITS (if any)

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Lab of Viral Carcinogenesis, NCI

LAB/BRANCH
Metabolism Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4 3/4	2 3/4	2

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☑ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The production of multiplication stimulating activity (MSA) by rat liver cell lines in tissue culture has been shown to be independent of the ability of these cell lines to multiply in serum free media. NSILA-s (non suppressible insulin-like activity) and somatomedin-A compete for MSA tracer binding to receptors in chick embryo fibroblasts, human skin fibroblasts, rat liver cells, and purified rat liver plasma membranes. MSA also binds to a wide variety of cells in culture including cell lines derived from human cancers. The binding pattern for MSA is different than the pattern for epidermal growth factor (EGF) binding. A somatomedin binding protein has been found in rat serum and the size of the binding protein and its serum concentration have been found to be growth hormone dependent. Somatomedin activity has been found to be low in several dwarf mice mutants (Snell, Ames and Little) and normal levels were found in the pygmy mouse.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The understanding of normal growth may be a prerequisite to understanding malignant growth. One experimental model for the study of normal growth has been the growth hormone treated hypophysectomized rat. It has been proposed that the anabolic action of growth hormone is mediated by a second circulating protein designated somatomedin. The object of this project is to purify somatomedin, develop in vitro tissue culture systems responsive to somatomedin and study the sequence of biochemical events accompanying growth.

Methods Employed: Somatomedin (MSA or multiplication stimulating activity) is being purified from the conditioned medium of rat liver cells in culture using ion exchange chromatography, gel filtration and preparative disc acrylamide electrophoresis. Serum starved cells in culture are being utilized to study the action of MSA. DNA synthesis is measured by incorporation of treated thymidine into DNA. Cell surface receptor and serum somatomedin binding protein studies utilize iodinated MSA as radioligand.

Major Findings: The original proposal that the rat liver cell line that produces MSA is able to multiply in serum free medium because it produces its own growth factor (MSA), was tested by studying MSA production in two other rat liver lines that multiplied in serum free media. Neither line was found to produce MSA although both cell lines (one a subclone of the MSA producer cell line) multiplied as well in serum-free medium as the producer cell line. We conclude that the ability of these rat liver cell lines to multiply in serum-free medium is independent of MSA production.

In collaboration with Matthew Rechler, Diabetes Branch, NIAMDD, human NSILA-s I and II (non suppressible insulin-like activity) and human somatomedin A have been studied in various MSA receptor systems (chick embryo fibroblasts, human skin fibroblasts, rat liver cell line and purified rat liver membranes). NSILA-s and somatomedin A were found to compete for MSA tracer binding to receptors in all these systems, providing further evidence for the relatedness of MSA to the human somatomedins.

In collaboration with George Todaso and Joseph deLarco, NCI, MSA receptors have been demonstrated in a wide variety of cells in culture including cell lines derived from human cancers. Certain human fibrosarcoma cell lines with low MSA binding are candidates for possible production of MSA or MSA related peptides. The binding pattern of various cells for MSA was found to be different than the binding pattern for epidermal growth factor (EGF).

Using iodinated MSA as radioligand a specific binding protein for MSA was found in normal rat serum. Tracer NSILA and somatomedin A both bound to the same size binding protein (as demonstrated by gel filtration chromatography) and were both displaced by excess unlabeled MSA suggesting that MSA binding protein could be called a somatomedin binding protein. A different sized binding protein was found in sera from growth hormone deficient rats (hypo-

physectomized). The size of the major serum binding component in hypophysectomized rats was shifted to the size of the protein found in normal rat serum by growth hormone treatment of the hypophysectomized rat. In addition, the level of the serum binding protein was found to be lower in the serum of the growth hormone deficient rat than in the normal rat and could be corrected toward normal by growth hormone treatment of the hypophysectomized rat. These studies, therefore, demonstrated that the serum somatomedin binding protein was growth hormone dependent. The MSA binding protein has also been detected in the conditioned media of two rat liver cell lines.

Mutant dwarf mice may be useful in studies directed at understanding the biochemistry of normal growth. Somatomedin activity levels were found to be low in three mutants with growth hormone deficiency (Snell, dw/dw, Ames df/df, and Little, lit/lit). On the other hand somatomedin activity was normal in another dwarf mouse, the pygmy (pg/pg), which was previously shown by others to be growth hormone resistant.

In collaboration with Brian Brewer (NHLI) the amino acid composition of MSA has been determined.

Significance to Biomedical Research: The understanding of the biochemistry and regulation of normal growth should provide a framework for understanding the abnormalities of growth regulation in malignancy. The elucidation of the growth peptides required for the growth of cells in tissue culture contribute to the eventual establishment of completely defined media for cells in tissue culture. Studies of the interaction of growth peptides with cell surface receptors define early steps in hormone action and may help in the elucidation of how this protein-membrane interaction leads to an anabolic response. Specifically, this research supports objective 3, approaches 1 and 2 and objective 4, approach 1 of the National Cancer Plan.

Proposed Course: We will continue to purify MSA from the conditioned media of rat liver cell line in collaboration with Matthew Rechler (NIAMDD) with the goal of accumulating sufficient material for sequencing.

The radioimmunoassay for MSA will be utilized to study the physiology of MSA in the rat including sites of synthesis and regulation of serum levels.

Regulation of MSA production by rat liver cells in culture will be studied in greater detail using the MSA antibody.

The rat and human serum binding protein for somatomedin (MSA) will be further characterized. The question of growth hormone dependence of the binding protein in man will be studied with a clinical protocol utilizing growth hormone deficient patients.

In collaboration with Wayne Anderson, NCI, the interaction of MSA with the ademylatecyclase system if being investigated.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CB-4017(c)-08-MET

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biology of the Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	Warren Strober, M.D.	Senior Investigator	MET	NCI
	Dean L. Mann, M.D.	Senior Investigator	IMM	NCI
	Howard B. Dickler, M.D.	Senior Investigator	IMM	NCI
	Pierre A. Henkart, Ph.D.	Senior Investigator	IMM	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Metabolism Branch

SECTION

Immunophysiology

INSTITUTE AND LOCATION

NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

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☑ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were undertaken to investigate the immunologic responsiveness of normal individuals and patients with immunologic disorders as well as experimental animals serving as models of human diseases.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Studies were undertaken to investigate the biology of immunologic responsiveness in normal individuals as well as patients with a variety of primary and secondary immunodeficiency diseases and disorders suspected of being the result of immunologically-mediated processes. Animal models of particular relevance to human diseases were also studied. Insight was sought into the mechanisms underlying perturbations of immunologic function present in these patients particularly those defects leading to an increased incidence of neoplasia.

Methods Employed: Emphasis was placed on defining the morphological and functional characteristics of various leukocyte and lymphocyte populations. For these studies, separation procedures based on the presence of certain surface receptors on only sub-populations of cells were employed to generate highly purified leukocytes and lymphocyte sub-populations. Techniques employed consisted of fractionation of cells over insolubilized immunoabsorbents including Sephadex G-200 anti-Fab, plastic-adherent anti-Fab, and plastic-adherent antigen-antibody complexes. Other cell separation techniques employed erythrocytes coated with various proteins, which, following interaction with cells bearing receptors for the specific proteins result in dense erythrocyte-lymphoid aggregates (rosettes) that may be separated from non-rosetting cells by buoyant density centrifugation. These separation techniques have been utilized in series and in parallel to yield highly purified sub-populations of cells for structural and functional analysis.

Morphologic analysis of mononuclear leukocyte sub-populations have included enumeration of macrophages by latex phagocytosis assays and staining for non-specific esterase. Bone marrow derived lymphocytes (B-cells) were enumerated by direct and indirect immunofluorescent staining for several cell surface characteristics including surface immunoglobulin (sIg), receptors for the Fc portion of complexed immunoglobulin (Fc receptors) and genetically controlled alloantigens (B-cell alloantigens-Ia alloantigens). B-cells were also enumerated by rosetting techniques employing IgM antibody and complement coated erythrocytes (complement receptors). Thymus-derived lymphocytes (T-cells) were enumerated by their receptors for sheep erythrocytes as well as by indirect immunofluorescence using alloantisera against thymic differentiation antigens (theta, Thy 1.2). Additional sub-populations of cells (K-cells) were defined on the lack of receptors characteristic of macrophages, B-cells and T-cells and the presence of high avidity Fc receptors.

The functional analysis of isolated leukocyte and lymphocyte sub-populations was primarily directed toward the definition of the cytotoxic effector potential of these cells. Use was made of a variety of target cell types in assays of "natural" or "spontaneous" cell mediated cytotoxicity (SCMC), mitogen-induced cellular cytotoxicity (MICC) and antibody-dependent cellular cytotoxicity (ADCC).

Major Findings: Cytotoxic Effector Cell Potential of Isolated Leukocyte and Lymphocyte Sub-Populations - Previous studies employing highly purified murine lymphocyte sub-populations and cells from congenitally athymic nude mice which demonstrated the critical importance of the target cells employed in assays of MICC and ADCC were extended to man. For these human studies purified peripheral blood neutrophils, macrophages, B-cells and T-cells were compared in assays of MICC and ADCC employing both chicken erythrocytes (CRBC) and allogeneic human Chang liver cells as targets. When CRBC were employed as targets, it was found that neutrophils, macrophages, T-cells, and B-cells could mediate MICC. In sharp contrast when Chang targets were used, only T-cells mediated MICC. With regard to ADCC, using CRBC targets, it was found that neutrophils, macrophages and K-cells could mediate cytotoxicity while with Chang targets only K-cells mediated ADCC. Thus in humans, when CRBC targets are employed in assays of MICC and ADCC, heterogeneous cell populations can effect cytolysis while when Chang targets are employed, MICC is T-cell mediated and ADCC is K-cell mediated. Additional studies were undertaken to examine species differences with regard to the antibody utilized to sensitive target cells for lysis in ADCC. With trinitrophenyl (TNP)-modified human erythrocytes as targets and human peripheral blood mononuclear cells as attackers, it was observed that rabbit but not goat anti-TNP sera were effective in cooperating to effect target cell lysis by K-cells. This occurred with the antisera being used at equivalent TNP-hemagglutinating titers. Thus in addition to the critical role played by the target cell, the species of anti-target cell serum employed in ADCC assays is of importance. Additional studies were undertaken to examine the phenomena of "natural" cytotoxicity or "spontaneous" cell mediated cytotoxicity (SCMC). Recently, a population of cells has been described in experimental animals which without known prior immunization is cytolytic to a variety of target cells in vitro. In experimental animals the presence of this spontaneous cytotoxicity is correlated with tumor resistance and a major role for this form of effector cell function may be in immune surveillance. An assay for SCMC employing Chang cell targets has been established in humans and the effector cell mediating SCMC, like the effector cell mediating ADCC was shown to be in the K-cell population lacking characteristics of macrophages, T-cells and B-cells. Studies revealed SCMC and ADCC effector cells to have identical organ distributions being present in spleen and peripheral blood and absent in tonsil. The mechanism of cytotoxicity for SCMC was demonstrated to be different than that for ADCC in that SCMC was inhibited by trypsin pretreatment of effector cells and this inhibition was not reversible by incubation in autologous plasma. Thus although cytolysis in both assays is mediated by K-cells, SCMC and ADCC were shown to involve separate cytotoxicity mechanisms which for SCMC probably does not involve "arming" in vivo with antibodies. Studies thus far are consistent with separate effector cells within the K-cell population mediating SCMC and ADCC or with a single effector cell mediating SCMC and ADCC via separate mechanisms. Employing Chang cell targets and having defined the effector cell mediating MICC as a T-cell and the effector cell(s) mediating SCMC and ADCC as K-cells, these cytotoxicity reactions were utilized to examine the functional capabilities of these lymphocyte sub-populations in patients with immunodeficiency diseases. In general, the cytotoxic effector cell capability of lymphocytes from groups of immunodeficiency

patients such as those with common variable hypogammaglobulinemia, dysgammaglobulinemia, ataxia-telangiectasia, the Wiskott-Aldrich Syndrome and selective IgA deficiency was normal. However, several major findings were made. With regard to MICC, a T-cell mediated reaction, cytotoxic effector cell capability of patients' lymphocytes was found not to correlate with other assays of T-cell function such as in vitro proliferation to T-cell mitogens and allogeneic cells or in vivo delayed cutaneous hypersensitivity. These findings again point out the heterogeneity and disparity of T-cell function in immunodeficiency disease patients as assessed by current in vitro and in vivo assays. With regard to ADCC and SCMC, the cytotoxic effector capability of patients' lymphocytes in these assays was very closely correlated. Patients lacking peripheral blood B-lymphocytes were found to have increased or diminished SCMC - ADCC effector cell activity which correlated with the number of peripheral blood K-cells as assessed by morphologic assays. Thus in certain patients with hypogammaglobulinemia K-cells may be present or absent, suggesting that abnormalities of lymphocyte differentiation in immunodeficiency disease patients also involve the K-cell sub-population. Chylous effusion lymphocyte populations from patients with intestinal lymphangiectasia and protein-losing enteropathy which are considered representative of human recirculating lymphocyte sub-populations contained T-cells but not K-cells and mediated MICC but not SCMC or ADCC. These results suggest that K-cells belong to a population of non-recirculating lymphocytes.

Studies of the Distribution of B-cell Alloantigens Genetically Linked to the Major Histocompatibility Complex - Recent studies in experimental animals and man have demonstrated the existence of polymorphic surface glycoproteins determined by genes closely linked to the major histocompatibility complex. These gen products are expressed on only sub-populations of lymphocytes and termed Ia antigens in mice and HLB antigens or B-cell alloantigens in humans. Studies were undertaken to examine the distribution of these alloantigens on various lymphocyte sub-populations. In humans these studies utilized a complement-dependent microcytotoxicity assay, multiparous pregnancy sera as a source of alloantisera, and lymphocyte sub-populations purified by a combination Sephadex G-200 anti-Fab and plastic adherent antigen-antibody complex fractionation. Specificities termed HLB antigens or B-cell alloantigens were found exclusively on sIg positive, Fc receptor positive B-cells and not on sIg negative, Fc receptor positive K-cells or a sub-population of sIg negative, Fc receptor negative T-lymphocytes. In contrast, HLA alloantigens were present on all lymphocyte sub-populations. These serologically defined B-cell specificities may be identical to or closely related to HLA-D locus antigens which may be of utmost importance in human organ transplantation. During the course of these studies it became apparent that a major limitation in histocompatibility typing for these alloantigens was the paucity of sIg positive alloantigen bearing cells in human peripheral blood (5-10% of lymphocytes). A major new discovery of extreme importance to histocompatibility typing was the development of a method whereby sIg positive, B-cell alloantigen positive lymphocytes could be bound to goat anti-Fab coated Micro-Test plates and tested for alloantigen specificities in situ. This advance will pave the way for the typing of various pateint groups for B-cell alloantigens using conventional microcytotoxicity techniques and lymphocyte sub-

populations enriched for alloantigen bearing cells. B-cell alloantigen typing was also applied to the study of immunodeficiency disease patients. It was observed that lymphocytes from patients with common variable hypogammaglobulinemia lacking sIg positive B-cells but possessing K-cells on both morphologic and functional criteria lacked cells bearing B-cell alloantigens while lymphocytes from other patients possessing sIg positive B-cells possessed B-cell alloantigen bearing cells. These histocompatibility typing techniques open new avenues for assessing defects in B-lymphocyte maturation in patients with immunodeficiency diseases as well as ascertaining the origin of malignant lymphocytes in patients with lymphoreticular malignancies.

In studies similar to those in humans and employing slightly different methodologies the distribution of Ia alloantigens was studied on isolated sub-populations of murine splenic lymphocytes. In these studies, alloantigens including Ia, Thy 1.2 and H-2, as well as sIg, and Fc receptors were studied by direct and indirect immunofluorescence. Using Sephadex anti-Fab immunoabsorbent fractionated macrophage-depleted splenocytes, the majority of sIg positive, Fc receptor positive B-lymphocytes were demonstrated to bear Ia alloantigens and the Fc receptors on such cells were associated with Ia antigens in that pretreatment of cells with anti-Ia sera inhibited binding of complexed immunoglobulin to Fc receptors. In sharp contrast, most sIg negative, Thy 1.2 negative, Fc receptor positive lymphocyte did not bear Ia alloantigens and the Fc receptor binding of complexed immunoglobulin on such cells was not blocked by prior anti-Ia treatment. Analysis of cytotoxic effector cell function in these cell populations revealed that K-cells capable of mediating ADCC were contained solely in the sIg negative, Ia antigen negative, Fc receptor positive sub-population. Furthermore, treatment of such cells with anti-Ia allosera and complement did not diminish ADCC effector cell activity. Thus a sub-population of Fc receptor positive lymphocytes capable of mediating ADCC was obtained and unlike sIg positive B-cells was shown to possess ADCC effector cell activity and lack Ia alloantigens. These results in mice with Ia alloantigens corroborate those studies of B-cell alloantigen distribution on lymphocyte sub-populations in humans and demonstrate further major differences between B-cells and K-cells.

Proposed Course: Studies using well characterized assays to evaluate in vitro cytotoxic effector cell function of patients with immunodeficiency diseases, autoimmunity, and neoplasia will be continued. Attempts will be made to correlate the observed in vitro cytotoxicity with the numbers of effector cells observed morphologically to ascertain if quantitative or qualitative abnormalities of lymphocytes can explain the differences in observed cytotoxic potential among patient groups. Particular emphasis will be placed on studies of "natural" or spontaneous cytotoxicity in patient groups known to be at increased risk of neoplasia in order to ascertain whether these patients like experimental animals with decreased tumor resistance, have reduced "natural" cytotoxic potential. Using morphologic criteria, additional studies will be carried out to assess abnormalities in the differentiation of lymphocyte sub-populations occurring in immunodeficiency diseases. Ongoing studies to determine the origin and functional capabilities of K-cells will be continued. In particular, attempts will be made to

further divide this sub-population in order to understand the observed differences in cytotoxic mechanisms for SCMC and ADCC shown to be mediated by this sub-population.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-ZO1-CBD-4018-01-MET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of Human Immune Defense Mechanisms and Its Control

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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4	2	2

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☑ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are aimed at understanding how the human immune system recognizes and destroys foreign cells. Further understanding should lead to new approaches in the therapy of infectious and neoplastic diseases.

PHS-6040

Project Description:

Objectives: The objectives of these studies were to 1) delineate the importance in man of a newly recognized system of non-specific cell mediated spontaneous cytotoxicity and 2) to investigate the immunoregulatory events involved in the maintenance of the human placenta. Both objectives are directly related to Objective 2, Approach 1 of the National Cancer Plan.

Methods Employed: In vitro cellular cytotoxicity systems have been developed for antibody dependent, mitogen induced, cell mediated lympholysis, and spontaneous cytotoxicity using a sensitivity micro ^{51}Cr release assay for various target cells. These results were correlated with standard in vitro and in vivo assays of cell mediated and humoral mediated immunity.

Our other main area of investigation involved the physico-chemical separation of pregnant urine using ion exchange chromatography, gel filtration, isoelectric focusing and gel electrophoresis. The resultant products were assayed for in vitro reactivity using standardized assays of cell mediated immunity.

Major Findings: Our continuing interest in various in vitro models of cell mediated cytotoxicity has led to several new insights regarding certain aspects of the cellular control of these phenomena. Previous work has shown that a multiplicity of human peripheral blood leukocytes can kill ^{51}Cr labelled targets given the appropriate specific or non-specific stimulus. Thus B cells, T cells, K cells, monocytes and polymorphonuclear leukocytes can kill all targets in vitro. Different results are then dependent upon the technical aspects of the assay, including 1) type of target, 2) source of stimulus (if any), 3) length of incubation, and 4) definition of cell death (i.e., dye exclusion, ^{51}Cr release, loss of adherence, etc.). Work is currently underway on a new model which examines the spontaneous in vitro generation of a potent class of non-specifically cytotoxic mononuclear cells. The mechanism by which these cells develop cytotoxic potential is dependent upon a time dependent loss of suppressor cell activity in vitro. Thus, freshly drawn mononuclear cells when assayed are not cytotoxic. However, after seven days of in vitro culture in the absence of any known exogenous stimulation the monocytes in these cultures develop profound and apparently completely non-specific cytotoxicity towards every red blood cell target tested to date. The addition of autologous freshly drawn lymphocytes to these seven day cultures suppresses killing down to baseline levels. The nature and mechanism of action of the suppressor cell is under active investigation. It appears to be a non-T cell. Furthermore, sonicated supernatants of freshly drawn cells contain a heat labile suppressor factor. Our expanding interest in this unique model stems from several observations. First, the model allows a potentially fruitful approach to study the negative regulatory influences which control cytotoxic monocyte function in vitro and in vivo. Second, this type of antigen non-specific non-immune monocyte killing is quite analogous to natural killing systems involving K cells and evidence is accumulating from numerous labs that this type of early non-specific killing may be a very important first line of defense against both infections

and malignant diseases. Third, we have discovered that our spontaneous killing model varies immensely with various disease states. For example, patients with acute viremia and certain patients with lymphomas are super killers and kill without the necessity of pre-culturing. Our data suggests that these patients lack circulating suppressors. Other patients, however, lack the ability to kill. Patients with CLL cannot kill due to a lack of circulating monocytes. The fact that patients with the Wiskott-Aldrich syndrome cannot kill is an independent confirmation of their previously described monocyte killing defect. Certain immunodeficient patients including a subset of common variable hypogammaglobulinemic patients and all patients with ataxia-telangiectasia fail to kill and the reasons for this deficiency are under active investigation.

Another area of great potential interest concerns circulating immunoregulatory substances which occur in the urine of pregnant women. Previous data had shown that commerical HCG contained an ideal non-toxic reversible suppressor of in vitro specific and non-specific lymphocyte responses. Furthermore our work has shown that HCG was not responsible but rather due to a contaminant found in commercial sources of HCG. Currently attempts at fractionating crude pregnant urine have discovered at least two and possibly three suppressive substances. Work is continuing on further characterization of these compounds.

Significance to Biomedical Research: These present studies extend our understanding of the way in which a host organism is able to cope with foreign grafts whether they are bacterial, malignant, or viral in origin. Our studies of non-specific spontaneous cytotoxicity suggest that it may play a central role in host defence and act long before specific mechanisms of immunity can be mobilized.

Our studies of the immunoregulatory events surrounding pregnancy offer the promise of understanding a central question of modern immunology and further may suggest new avenues of approach in such diverse fields as organ transplantation or infertility.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4019(c)-08-MET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunoregulatory Cells in Human Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
Metabolism Branch

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NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate the immunoregulatory properties of malignant lymphocytes. We are currently determining the helper and suppressor activity of neoplastic T-cells from patients with acute lymphoblastic leukemia and the Sézary syndrome.

PHS-6040

Projection Description:

Objectives: A number of critical insights into the nature of the humoral immune response were made possible by studying neoplasms of the B cell/plasma cell series. The recognition that the so-called paraproteins derived from patients and certain animals with myeloma (a malignant proliferation of the plasma cells) represented extremely homogeneous immunoglobulins has affected virtually every phase of immunologic research. Our understanding of antibody structure, function, and genetics depends heavily on experiments which use such homogeneous immunoglobulins. Indeed, certain immunoglobulin classes such as IgD and IgE would have been difficult or impossible to study without the availability of the appropriate paraprotein classes.

Recently recognized membrane and functional differences between B cells and T cells have provided an important basis for classifying neoplastic lymphocytes. Such a classification has already proved to have therapeutic and prognostic relevance in characterizing lymphomas and certain leukemias. The study of malignant T cells and their products may prove to be as exciting in answering questions regarding cellular immunity and the T cell regulation of humoral immune responses as myeloma cells and their paraprotein products have proved to be in answering questions concerning the immunoglobulin synthesizing system. During the past year we have focused considerable research and clinical effort in studying patients with the Sézary syndrome. The hallmarks of this grave disorder are an exfoliative erythroderma, generalized lymphadenopathy and circulating malignant lymphocytes with the propensity to infiltrate skin. These circulating neoplastic lymphocytes, referred to as Sézary cells, have a deeply folded or cerebriform nucleus as their key morphologic feature. A number of workers have shown that Sézary cells from most patients have a T-cell origin.

T cells play a critical role in a number of immune systems. These cells are involved in cell-mediated immunity which includes such phenomena as delayed hypersensitivity, allograft rejection, and graft vs. host reaction. Normal T cells proliferate in vitro when stimulated with a number of plant mitogens such as phytohemagglutinin and conconavalin A. They may mediate the mixed leukocyte reaction, act as killer cells in certain models of leukocyte induced cytotoxicity, and generate certain soluble effector proteins known as lymphokines. In addition, T cells play an indispensable role in the regulation of humoral immune responses by acting as potentiators (helper cells) or inhibitors (suppressor cells) of the transition of B cells into immunoglobulin producing plasma cells. Both antigen-specific and non-specific helper and suppressor function have been identified.

The leukemic T cells of patients with the Sézary syndrome have been examined by other workers for their capacity to mediate certain of the normal T cell function involved in cell mediated immunity. The basic conclusion is that Sézary cells from various individuals differ in the degree of normal T cell activity they may still express. Thus, the leukemic cells of some patients responded normally to plant mitogens while the cells of the majority of the patients responded poorly or not at all. The Sézary cells from most of the patients did not transform normally to performed blastogenic factor. Leukemic cells from a few patients functioned as both effective stimulators and responders in mixed lymphocytes reactions, whereas the cells from others were either weak stimulators, weak responders or both. Sézary cells from most patients were poor

killers in assays of T-cell mediated cytotoxicity. On the other hand, the majority of Sézary syndrome patients had leukemic cells that could produce a lymphokine resembling migration inhibition factor. The capacity of the Sézary cells to act as helper cells or suppressor cells in humoral immune responses has not been investigated by other workers. The purpose of this study was to determine whether Sézary cells could regulate the in vitro synthesis of immunoglobulins by lymphocytes derived from normal individuals and from patients with thymic deficiency states.

Methods Employed: To study the transition of circulating lymphocytes into immunoglobulin secreting plasma cells, peripheral blood lymphocytes were cultured in the presence of pokeweed mitogen. The amount of IgM, IgA and IgG synthesized and secreted in the medium was determined by double antibody radioimmunoassays of these immunoglobulins. The presence of circulating suppressor cells was assayed by a co-culture technique. Peripheral blood Sézary cells from patients and peripheral blood lymphocytes from controls were cultured together. The synthesis of immunoglobulin by cells of the two subjects in co-culture was related to the sum of the expected contribution by each population. In order to assay helper cell activity we took advantage of the observation that highly purified normal B cell populations, freed of T cells, synthesize very small quantities of immunoglobulin. The ability of added cells to augment immunoglobulin synthesis by these highly purified B cells was used as a test for helper cell activity.

Major Findings: The neoplastic lymphocytes from patients with the Sézary syndrome did not synthesize significant amounts of the immunoglobulins. In addition, they did not show suppressor cell activity in co-culture with normal lymphocytes. Indeed, on a number of occasions normal lymphocytes showed a greatly amplified immunoglobulin synthesizing and secreting capacity in the presence of Sézary cells compared to their capacity when cultured alone. In addition, the circulating lymphocytes from 7 of 10 patients with the Sézary syndrome studied functioned as helper T cells. Thus, there was a 6 to over 30-fold augmentation of immunoglobulin production by normal purified B cells after the addition of Sézary cells. Furthermore, Sézary cells from one patient studied induced a nearly 10 fold increase in IgA synthesis by lymphocytes from a child with ataxia telangiectasia and isolated IgA deficiency. Moreover, these Sézary cells induced more than a 500 fold increase in IgG and IgA synthesis by lymphocytes from a child with Nezelof's syndrome. Both children had diseases characterized by severe thymic dysfunctions. A series of control studies were carried out to determine if helper cell function was a specific property of cells from patients with Sézary syndrome or was merely the result of co-culturing allogeneic cells. In these studies, it was found that culturing the malignant lymphocytes from patients with chronic lymphocytic leukemia, a amlignancy of B-cells or cells of the long term-B-cell tissue culture lines NC37 and PA3 did not augment immunoglobulin synthesis by purified normal B cells. Similarly, the long term T cell line Molt 4 did not mediate helper cell activity. This supports the view that the mere co-culture of allogeneic cells does not lead to apparent helper cell activity.

One of the most critical questions concerning the T cell regulation of immunoglobulin synthesis is whether a single population of T cells can mediate

both helper and suppressor activity under appropriate conditions or whether help and suppression are accomplished by 2 distinct classes of T cells, each genetically programmed to carry out only helper or suppressor type function. Our results over the past year are in accord with the view that human helper cells are distinct from human suppressor cells. Our conclusion that Sézary cells represent a homogeneous expansion of helper cells in perhaps most clearly supported by experiments which involved adding graded increases of normal T cells to a constant number of normal B cells. When progressively more normal T cells were added to purified normal B cells suppression of pokeweed mitogen immunoglobulin production was observed at high normal T cells to B cells ratios. At least one explanation for this is that normal circulating lymphocyte populations contain both helper and suppressor T cells. In contrast, there was a progressive increase in the quantity of immunoglobulins synthesized by normal B-cells when increasing numbers of Sézary cells were added. In general, there was no evidence of decline in immunoglobulin synthesis at very high neoplastic B cell to T cell ratios. Furthermore, the addition of a constant number of Sézary cells to mixtures of normal B cells and normal T cells prevented the decline of immunoglobulin production caused by excessive numbers of normal T cells.

We next turned our attention to a select group of patients with the Sézary syndrome who had low numbers of circulating neoplastic T cells. This select group differed from the previous patients studied in that the absolute number of neoplastic lymphocytes in the peripheral blood was less than 2000 cells/mm^3. The Sézary patients discussed above had very high leukemic counts, often in the range of 50,000-100,000 malignant cells/mm^3. If our conclusion that the Sézary syndrome represented a leukemia of cells programmed for helper activity was correct, one might expect that the circulating lymphocytes in patients with the low count Sézary syndrome would show excessive production of those immunoglobulin classes which appear to be highly thymic dependent. In other words, the patients' own Sézary cells should promote the terminal maturation of autologous B cells. This in fact appeared to be the case. The lymphocytes of 5 patients with the low count Sézary syndrome showed excessive production of IgA, a highly thymic dependent immunoglobulin class in the absence of pokeweed mitogen stimulation. In addition 2 of 4 patients examined showed an abnormally high number of circulating plasma cells demonstrated by electron microscopy. Thus, in patients with the low numbers of circulating neoplastic T cells, the autologous B cells appear to be abnormally programmed in vivo for excessive immunoglobulin production. This concept is strengthened by the observation that the IgA concentration in the serum of such patients is significantly elevated.

A major effort of the Metabolism Branch centers around developing new diagnostic and therapeutic approaches which may enhance the clinical care of patients admitted for immunologic research purposes. Patients with the Sézary syndrome have an extremely poor prognosis and may be critically ill by the time they are referred to the Metabolism Branch. Accordingly, a collaborative program has been established between the Metabolism Branch and the Division of Cancer Treatment (Medicine Branch and Veterans Administration Hospital). All patients admitted undergo a thorough lymphoma staging protocol on the Metabolism Branch. This staging workup generally includes a liver biopsy and lymphangiogram. In collaboration with the Division of Cancer Treat-

ment, those patients with malignant lymphadenopathy and/or visceral involvement are treated with combination chemotherapy and electron beam radiotherapy. In a recently devised pilot protocol, patients who fail conventional chemotherapy and electron beam radiotherapy will begin a regimen of heterologous anti-human thymocyte globulin administration. One patient has already received anti-thymocyte globulin and showed dramatic reduction of tumor load by both physical examination and lymphangiography.

Significance to Biomedical Research. The recognition that the Sézary syndrome represents a homogeneous proliferation of T cells which have a helper-like function is important for a number of reasons. First, certain forms of humoral immune deficiency may be due to a helper cell deficiency and not only due to an intrinsic defect of B cells alone or excessive numbers of suppressor cells. It is possible that certain classes of neoplastic T cells or soluble products produced by such cells could prove useful in the treatment of immune deficiency states due to thymic dysfunction. Similarly, if such helper cell factors could be identified and synthesized, they might be useful in those situations wehre it is desirable to amplify a normal immune response such as following certain immunizations or in overwhelming infections. Second, in mice it has been possible to show that the genetically controlled Ly system of antigens in mice denote cell surface markers for helper and suppressor functions. Three groups of T cells have been identified in mice. One population bearing all 3 of the so-called Ly determinants (Ly 123 positive), one population bearing Ly1 determinants alone, and a third population bearing Ly23 determinants. It has been demonstrated that those cells that are Ly1 positive act as helper cells. In contrast, those cells which are Ly23 positive act as suppressor cells. The use of Sézary cells may make it possible to establish a comparable marker system for normal and neoplastic human lymphocytes. Finally, the study of patients with the Sézary syndrome may provide a clinical opportunity to test the feasibility of using anti-neoplastic cell antisera alone or in combination with systemic chemotherapy or radiotherapy. It is currently unclear whether antisera directed against neoplastic cells have any real clinical role. In most human tumor systems, it is extremely difficult to obtain appropriately specific antisera. The Sézary syndrome provides a clinical model of a tumor with relatively distinct cell-surface antigenic properties. Heterologous antisera capable of reacting with and theoretically destroying Sézary cells are currently available. Moreover, patients with the Sézary syndrome have a number of easily measured parameters of tumor growth or regression (e.g. skin involvement, clinically palpable lymph nodes, lymph nodes seen on lymphangiography and circulating number of neoplastic cells) which make it possible to objectively document any therapeutic benefit from the use of heterologous anti-lymphocyte antisera. If heterologous antisera are found useful for this disease, it might provide a rational basis for using appropriate antisera in other tumor systems.

Proposed Course: During the coming year a continuing effort will be made to characterize the immunoregulatory properties of lymphocytes from patients with T-cell leukemias and lymphomas. A very high priority will be given to the investigation of the T-cell variant of acute lymphoblastic leukemia. We have preliminary data to suggest that some children with acute lymphoblastic leukemia may have malignant T cells with suppressor properties. One goal will be to investigate the cell membrane properties of such neoplastic T cells using established markers, such as the capacity to form so-called IgM-rosettes and IgG-rosettes. In addition, we will attempt to use neoplastic lymphocytes to raise heterologous antisera which can be used to categorize normal T cells on the basis of helper or suppressor function. We will also investigate whether it is possible to isolate soluble factors from malignant lymphocytes which can mediate the immunoregulatory properties of intact cells. Furthermore, we will continue our clinical investigation into the therapeutic usefulness of horse anti-thymocyte globulin in selected patients with malignancies which have a T cell origin.

Publications:

Waldmann, T.A., Broder, S., Durm, M., Meade, B., Krakauer, R., Blackman, M., and Goldman, C.: T-cell suppression of pokeweed mitogen induced immunoglobulin production. In Role of Mitogen in Immunobiology. Oppenheim, J.J. and Rosenstreich, D. (Eds.). Academic Press, Inc., New York. 509-521, 1976.

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Iio, A., Strober, W., Broder, S., Polmar, S., and Waldmann, T.A.: The metabolism of IgE in patients with immunodeficiency states and neoplastic conditions. J. Clin. Invest. 59:743-755, 1977.

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Broder, S., Dobbins, W.O., and Waldmann, T.A.: Excessive IgA production by lymphocytes from patients with the Sezary syndrome and low numbers of circulating neoplastic T cells. Fed. Proc. 36:1210, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER NCI-Z01-CBD-4020(c)-08-MET

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetic Control of the Immune Response to Natural Protein Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jay A. Berzofsky, M.D., Ph.D.	Expert	MET	NCI
OTHER:	David H. Sachs, M.D.	Senior Investigator	I	NCI
	David Pisetsky, M.D., Ph.D.	Clinical Associate	I	NCI
	Ronald H. Schwartz, M.D., Ph.D.	Senior Investigator	LI	NIAID
	Alan N. Schechter, M.D.	Senior Investigator	LCB	NIAMDD

COOPERATING UNITS (if any)
Immunology Branch, NCI
Laboratory of Immunology, NIAID
Laboratory of Chemical Biology, NIAMDD

LAB/BRANCH
Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Genetic control of the antibody and T-cell proliferative responses of mice to the natural protein antigens staphylococcal nuclease and sperm whale myoglobin has been studied. H-2 linked control of the response to nuclease has been shown to be exerted at the level of individual determinants independently of one another, rather than the molecule as a whole, by comparing the specificity of antibodies made to nuclease by two different pairs of congenic mice, each differing only at H-2. T-cell proliferative studies have suggested the existence of more than one H-2-linked Ir gene controlling the response to the several determinants. In addition, a non-H-2 linked gene(s) was demonstrated to control the overall level of response to the whole molecule, and was shown to be unlinked to heavy chain allotype and sex as well. Finally, the antibody response to myoglobin was demonstrated to be under control of at least two genes mapping in different Ir subregions of the H-2 complex, as well as at least one non-H-2-linked gene.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Since Ir (immune response) genes have been studied primarily in the response to synthetic polypeptides or artificial hapten-carrier antigens, it was felt useful to study the fine specificity of the control of the response to natural globular proteins, which differ from most of the above by having a unique, nonrepeating set of determinants with no obvious "hapten" or "carrier" portions. By elucidating the specificity of control it was hoped to better understand the mechanism of action of these Ir genes, which appear to duplicate, at the regulatory level, the antibody's function of antigen recognition. In addition, it is hoped to develop systems in which the cell membrane receptors involved in this control can be studied.

Methods Employed: Mice of a variety of strains in several congenic resistant series were immunized i.p. with a given protein antigen or polypeptide fragments of that antigen in complete Freund's adjuvant and boosted i.p. with antigen in saline. Successive sera obtained from these mice were analyzed individually or in pools for concentration of antibodies which could bind to the immunogen or its fragments by a radiobinding assay. The proteins or peptide fragments were labeled at the N-terminal amino group by carbamoylation with $K^{14}CNO$. Increasing concentrations of labeled antigen were allowed to react with a constant concentration of serum, and bound antigen was separated from free antigen by precipitation of the immunoglobulin with polyethylene glycol. The concentration of specific antibody was then obtained from the plateau of the saturation binding curve, and the affinity could be obtained from a Scatchard plot. T-cell proliferation of Peritoneal Exudate T-lymphocyte-Enriched Cells (PETLES) from primed mice was assessed by in vitro incubation of these cells with increasing doses of stimulating antigen and measuring incorporation of a pulse of ^{3}H-thymidine into DNA at the end of the culture period. The proteins and fragments obtained from them were prepared and purified by published methods.

Major Findings: In collaboration with Drs. D. Pisetsky and D.H. Sachs, sera from mice immunized with whole native nuclease were studied for their ability to bind labeled fragments of nuclease. As we had found for the congenic pair B10 and B10.A strains, mice of the strains A/J and A.BY were demonstrated to produce comparable amounts of antibody to one region of nuclease but very different amounts to another region of nuclease, demonstrating that clearly H-2-linked genes could control the response to different determinants separately rather than to the molecule as a whole. In addition, non-H-2-linked genes were identified which did control the level of antibody in aggregate to the whole molecule. This gene or genes which produced a higher response in "A background" strains than in "B10 background" strains of the same H-2 type, was found to segregate independently of heavy chain allotype (structural genes) and of sex in a (B10.A x A/J) x B10.A backcross. The distribution was not inconsistent with single gene control. In addition, the same or another non-H-2-linked gene differing in these strains led to a 10-fold difference in antibody affinity to the fragment of nuclease (1-126) for which the H-2-linked genes were less dominating. The higher responders (A background) produced the lower affinity antibodies.

Dose-reponse curves (in collaboration with Drs. R.H. Schwartz, D.H. Sachs, and A.N. Schechter) for the proliferative response of T-cells from nuclease-primed mice stimulated with fragments of nuclease demonstrated similar fine

specificity of the response as found for antibodies. Thus, such specificity does not require B cells. Moreover, study of a series of intra-I-region recombinants produced evidence for the existence of more than one Ir gene mapping in different I subregions (probably I-A and I-C) of the H-2 complex involved in the control of the response to the several determinants of nuclease. Also, participation of the I-J subregion genes, associated with suppressor function, was ruled out.

To develop a more flexible system to extend these studies, the immune response to myoglobin was studied for genetic control in mice. H-2-linked control of the antibody response was clearly demonstrated, with two high-responder haplotypes and three low-responder haplotypes, studied in two series of congenic resistant mice on different backgrounds. The control was mapped to the I region of the H-2 complex. Evidence was obtained for the existance of at least two distinct Ir genes mapping in different I subregions (I-A and I-C or to the right of I-C) controlling the response. In addition, a non-H-2-linked genetic influence was also demonstrated.

Significance to Biomedical Research: The demonstration that H-2-linked Ir genes can control the response to individual determinants on an antigen molecule separately, rather than the molecule as a whole, disproves one of the major widely accepted models in which H-2-linked Ir gene control is exerted at the level of T-cell recognition of a carrier part of the molecule, which would then allow T-cell help to B cells recognizing any determinants on the molecule. On the other hand, the demonstration of a non-H-2-linked gene(s) which regulates the aggregate level of all the antibodies to all the different parts of the molecule, independent of their relative proportions, sheds light on a different mode of regulation of the immune response. This control on the sum of different antibodies may be analogous to that seen in idiotype suppression, in which other idiotypes increase to keep the total level of antibody to a given antigen constant. The idea of different genes in different subregions controlling the response to different determinants explains the difficulty in detecting Ir genes to most complex antigens, and suggests that such control may be virtually universal if individual determinants rather than whole molecules are studied. The observation of similar determinant control of T-cell proliferation in the absence of B cells emphasizes the apparent lack of separate carrier and hapten function in this globular protein antigen. The new myoglobin system promises to open new possibilities for study of the intricate mechanisms of genetic control of immune responsiveness. It is hoped that these will lead to a better understanding of diseases in which the immune response is defective, as in malignancy, or overactive, as in autoimmune diseases, in an antigen-specific fashion.

Proposed Course: The genetic control of antibody affinity is to be tested for allotype linkage in the nuclease system. The intra-H-2 recombinants in which possible complementation was observed in T-cell proliferation will be studied at the antibody level, along with their F_1 hybrid. An F_2 generation of (B10 x A/J) mice is being selected for homozygous Iglb/1b allotype mice, and the $H\text{-}2^a$ positive ones of these will be studied for antinuclease antibody specificities to rule out genetic drift in the Ig structural genes between the supposedly congenic B10 and B10.A as an explanation of some of the above.

Acetylated bovine serum albumin is being prepared to see if attaching nuclease to a "carrier" molecule can bypass some of the levels of control, and in the process rule out determinant-specific suppression. Blocking studies using anti-Ia and anti-antigen sera to block the in vitro proliferative response to nuclease and its fragments are underway. These may allow further gene mapping, as the sera can be made quite specific.

Fragments of myoglobin can be used to determine whether the two H-2 linked Ir genes in fact control the responses to different determinants, as predicted. Also, myoglobins from other mammalian species, which differ at only a few amino acid residues, can be used to map determinants in a fashion not available for nuclease. The heme of myoglobin may be used as a probe for biophysical techniques. Hopefully, myoglobin will also serve in planned studies of membrane receptors for antigen.

Publications:

Berzofsky, J.A., Schechter, A.N., Shearer, G.M., and Sachs, D.H.: Genetic control of the immune response to staphylococcal nuclease III. Time course and correlation between the response to native nuclease and the response to its polypeptide fragments. J. Exp. Med. 145:111-122, 1977.

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Sachs, D.H., Berzofsky, J.A., Fathman, C.G., Pisetsky, D.S., Schechter, A.N. and Schwartz, R.H.: The immune response to staphylococcal nuclease: A probe of cellular and humoral antigen-specific receptors, in Cold Spring Harbor Symposium on Quantitative Biology, 41:295-306, 1976.

Berzofsky, J.A., Schwartz, R.H., Schechter, A.N., and Sachs, D.H.: H-2 linked control of the antibody and cellular immune response to nuclease at the level of individual regions of the molecule. In Symposium Proceedings of the Third Ir Gene Workshop. H.O. McDevitt, (Ed.). 1977, in press.

Berzofsky, J.A., Schwartz, R.H., Schechter, A.N., and Sachs, D.H.: T cell proliferative and antibody responses to nuclease in mice: H-2-linked control at the level of individual determinants. J. Supramolec. Struc., Suppl. 1:179, 1977 (Abstract).

SUMMARY REPORT
LABORATORY OF PATHOLOGY
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE
July 1, 1976 to September 30, 1977

The diagnostic and research activities of the staff of the Laboratory of Pathology are listed by Sections. The research accomplishments are especially impressive when one considers that the majority of the staff participate in the diagnostic services for Clinical Center patients. In fact, because of changes in professional personnel and additional administrative responsibilities, the overall diagnostic workload of several members of the staff has increased appreciably during the past year. Dr. T.A.S. Hanson resigned from his position as an Expert Pathologist in December, 1976. His duties as Acting Head of the Surgical Pathology and Postmortem Section were assigned to Dr. José C. Costa. These duties include supervision of the residents and of all staff pathologists who participate in the surgical pathology and autopsy diagnostic services. Dr. Bruce L. Webber joined Dr. Costa's service as a Visiting Scientist in January, 1977. These personnel changes have improved the diagnostic services because both Dr. Costa and Dr. Webber are expert diagnostic pathologists and are excellent teachers. Dr. Gregory T. O'Conor, Associate Director for International Affairs, NCI, has accepted a dual appointment as a Staff Pathologist and will participate in diagnostic studies. In March, 1977 the Image Processing Unit was transferred to the Quantitative Cytology Section. It is by offering opportunities and facilities for laboratory research that we have been able to recruit and retain an outstanding group of anatomical pathologists who have given vital support to the clinical programs of NCI as well as all the other Institutes with patients in the Clinical Center.

The research programs of the Laboratory have involved:

A. Hematopathology and Immunopathology
B. Viral Oncology and Cell Biology
C. Comparative Pathology and Carcinogenesis
D. Development and Use of Automated Equipment for Cytology Diagnoses
E. Clinico-Pathologic Studies on a Variety of Human Diseases

Some of the significant research and clinico-pathologic accomplishments in which the staff of the Laboratory participated, either as principal investigators or as collaborators include:

(1) Previous experimental work showed that neurovirulent strains of Type 1 polioviruses could be differentiated from attenuated, non-virulent strains by the structural lability of virion polypeptides (PV1). Further experiments indicate that increased neurovirulence in poliovirus strains recovered from the feces of children fed oral vaccine is due to back-mutation of the virus to manufacture structurally normal, stable VP1 polypeptide. Additional observations that vaccine-associated paralytic poliomyelitis in immunodeficient children is also probably due

to the lack of neutralizing antibodies in the immunologically defective patients.

(2) Further characterization of the morphology, immunoglobulin production and surface membrane characteristics of cells of human lymphomas, particularly the cells of "hairy" cell leukemia and the presence of distinctive lesions, pseudosinusoids in the spleen and angiomatous structures in the liver, associated with "hairy" cell leukemia.

(3) Pathologic and statistical studies of reviewed breast cancer cases from the HIP Breast Cancer Study and the relationship of these findings to the assessment of risks and benefits of including mammography in the routine screening of asymptomatic populations of women for breast cancer.

(4) Development of experimental and mathematical models for the study of metastases in animals. Demonstration that access of fibrosarcoma cells to the circulation and the number of metastases may be suppressed by BCG stimulated peritoneal macrophages inoculated intravenously.

(5) Demonstration that polyoma virus transformed epithelial cells cultured on millipore filters in the absence of mesenchyme show different types of differentiation and biological behavior than do similar transformed cells cultured in the presence of mesenchyme. The development of this and other types of experimental models for the interaction of epithelium and mesenchyme will be used to study factors which influence differentiation, morphogenesis and neoplastic transformation.

Many members of the Staff, in addition to their laboratory and service work, serve on numerous national and international committees, editorial boards, etc. Some of the principal activities of this sort are: Dr. C.J. Dawe, Project Officer for the Registry of Tumors in Lower Animals (Smithsonian Institution); Dr. C.J. Herman, Co-chairman of the DCBD Committee on Cytology Automation; Dr. L.B. Thomas, WHO Committee to develop and publish the International Classification of Diseases for Oncology (ICD-O), member of Council International Academy of Pathologists (USA-Canadian Division), Chairman of NCI Pathology Working Group to review breast cancer, member of ad hoc committee to review osteogenic sarcoma treated by interferon (Stockholm); Dr. C.W. Berard, member of USA Lymphoma Panel and faculty of 9th National and 2nd International Tutorials on Neoplastic Hematopathology, Editorial Board of Laboratory Investigation, Project Officer for pathology group review of non-Hodgkin's lymphomas, Regional Pathologist for the Eastern Cooperative Oncology Group; Drs. Elaine Jaffe and Raul Braylan, faculty members of Tutorials on Neoplastic Hematopathology; Dr. D.E. Henson, Short Course on Pathology of Virus Infections, IAP, and member of College of American Pathologists committee which developed and published the Systematized Nomenclature of

Medicine (SNOMed) and WHO committee to develop and publish ICD-O; Dr. E.W. Chu, faculty member of Tutorial on Clinical Cytology and Diagnostic Cytology Symposium, collaborative study on cytologic screen for bladder cancer with Cairo Cancer Institute; Dr. N.A. Young, attending physician for infectious disease consultation services in the Clinical Center and National Naval Medical Hospital; Dr. M.F. Stanton, member of FDA Product Advisory Committee for Toxicology of Specific Drug Contaminants and NRC Committee on Histologic Classification of Laboratory Animal Tumors; Dr. Thomas, Dr. Rabson and staff, lecture series in pathology to Georgetown University medical students.

Dr. A.J. Garvin who completed residency training in June, 1976 and who has been working with Dr. E. Neufeld from July, 1976 to June, 1977 will rejoin the Laboratory as a junior Staff Pathologist in July, 1977. He will be assigned to the Ultrastructural Pathology Section and will set up a histochemistry laboratory and participate with Dr. Costa and Dr. Triche in the diagnostic pathology services. Dr. Robert Reddick will complete residency training in June, 1977 and will also transfer to Ultrastructural Pathology as a junior Staff Pathologist. He will work with Dr. Triche and be primarily responsible for diagnostic E.M. Dr. Peter Howley will finish residency training in June, 1977 and will transfer to the Viral Oncology and Molecular Pathology Section under Dr. Young. He will continue his research and also participate in diagnostic pathology. In July, 1977 Dr. David Zopf will transfer from NIAMDD to the Ultrastructural Pathology Section under Dr. Triche. He will be an Expert Pathologist and will conduct research and participate in the diagnostic services. Dr. Raul Braylan will resign his position in Hematopathology at the end of July to take a position in pathology at the University of Florida Medical School. Dr. Risa Mann will complete her 2 year Staff Fellowship in Hematopathology in June, 1977 and will return to Johns Hopkins University School of Medicine. Dr. Koji Nanba completed his Fogarty International Research Fellowship in Hematopathology and returned to the University of Hiroshima in September, 1976. Dr. Marie Ricard, also under Dr. Berard in Hematopathology, completed her 1 year assignment as a Guest Worker and returned to the Centre Hospitalier et Université de Creteil in September, 1976. Dr. Yuji Kurokawa will complete his second year as a Visiting Scientist (Fogarty International Research Fellow) with Dr. C.J. Dawe and return to Tohoku University, Sendai, Japan in June, 1977. Dr. Nadia Bourgeois from the Universitaire Instelling Antwerpen (Belgium) spent 3 months as a Guest Worker with Dr. Berard from December, 1976 to February, 1977. Dr. Oliver Alabaster has an Expert position with Dr. Herman in Quantitative Cytology. Dr. Yoshiko Nakamura is a Visiting Fellow with Dr. Young in the section of Viral Oncology and Molecular Pathology.

In July, 1977 Dr. Jeffrey Cossman, University of Michigan Medical School, and Dr. Thomas Callihan, George Washington University Medical School, will join the Hematopathology Section in IPA positions. Dr. Unnur Thorgeirsson from George Washington University will join Dr. Young in VOMP in an IPA position. Ms. Francoise Rentier-Delrue started work with Dr. Young as a Visiting Associate in the VOMP Section. Dr. Stuart A. Bentley from Royal

Postgraduate Medical School will be a Visiting Scientist in the Quantitative Cytology Section starting July, 1977 and will work with Dr. Lewis Lipkin and staff in the Image Processing Unit.

The Staff were either senior authors or coauthors of 66 papers published in 1976. Thirteen papers have been published through April, 1977 and there are currently more than 40 papers in press and 58 papers submitted for publication.

Surgical Pathology and Postmortem Service

Dr. José Costa together with Dr. Bruce Webber and the residents and other pathologists from the Laboratory of Pathology plus Dr. R. Friedman (NIAMDD), Dr. A. Cheever (NIAID), Dr. W.C. Roberts (NHLI), Dr. Robert Stern (NIDR), Dr. K. Stromberg (NCI) were responsible for the surgical pathology and autopsy diagnostic services. Approximately 7140 surgical specimens or biopsies were accessioned during 1976; 2441 were bone marrows; 244 autopsies were accessioned; 71 of these were submitted. These specimens involved the preparation of more than 61,000 slides including nearly 44,000 H & E stained sections, more than 7500 special stains and 468 frozen sections.

Many specimens of fresh human tissues, including the eyes which are regularly removed during a complete autopsy, are furnished to NIH scientists in various laboratories.

Conferences with individual clinicians and surgeons from the various institutes are an important part of the care and study of patients in the Clinical Center. Regular gross specimen reviews of organs removed at autopsy are conducted by the staff and most surgical specimens are discussed at the weekly Surgical Pathology Conference conducted by Dr. Costa, Dr. Webber and the residents and staff. In addition there are other regular conferences including the Hematology-Lymphoma Conference (Drs. Berard, Jaffe, Braylan, and Mann), the Pediatric Oncology Conference (Dr. Triche), and a monthly Neuropathology Conference (Dr. Valsamis), and the Sarcoma Staging Conference (Drs. Costa and Webber). The staff and residents present selected autopsies at numerous clinico-pathologic conferences (e.g. NCI - Dr. Costa, NHLI - Dr. Roberts). A weekly seminar for presentation of research by the pathology staff has been organized by Dr. Herman.

During the year Dr. Costa has spent considerable effort to complete the reorganization, started by Dr. T.A.S. Hanson, of the Surgical Pathology and Postmortem services so that the quality of services rendered will be maintained, improved and made more efficient. These efforts include, among others, the teaching of residents to perform frozen sections on surgical specimens, providing better service for frozen sections to the neurosurgeons, expediting the preparation, filing and binding of pathology reports, introducing plastic embedding and thin sectioning as a routine for some tissue specimens and working with Dr. Triche and Dr. Reddick to increase the use of electron microscopy in diagnostic pathology.

Laboratory of Pathology, DCBD, NCI

The residents, in addition to duties in diagnostic pathology, have active research programs including clinicopathologic studies and laboratory experiments. Some of their experimental studies are listed under specific sections; e.g. the work of Dr. Howley and Dr. Strauchen in VOMP and Dr. Reddick's work in Ultrastructural Pathology. Dr. Lance Liotta has been especially productive in research related to experimental metastases. Published studies or studies submitted for publication include observations on degradation of basement membranes by metastasizing tumor cells, development of mathematical models for quantitation of tumor cell invasion rate and removal rate in the target organ for metastases, and immunologic suppression of fibrosarcoma tumor cell metastases by BCG stimulated macrophages. Dr. Jan Orenstein, who will finish residency in July, 1977 and will then take a position in the Department of Pathology of George Washington University Medical School, has continued his studies in collaboration with Dr. E. Shelton on the surface characteristics of murine lymphocytes and macrophages and the effects of temperature and antimetabolites on these surface characteristics. He has also participated as a coauthor in the publication of studies on the differential response of type-C and intracisternal type-A particle markers in cells treated with iododeoxyuridine and dexamethasone. During the year, Dr. W. Cook has done experimental work in Dr. Robert Friedman's laboratory on in vitro transcription of AAV genome with purified eucharistic RNA polymerase and studying the integration of viral genomes in human cells.

Publications of Studies on Human Material

Approximately half of the 1976 publications by the staff and residents involved human tissues or some aspect of human disease. These include flow microfluorometry for screening cervical and vaginal cytologic material (Herman-3); cell surface properties in Sezary syndrome (Braylan-7) and in cells of other types of malignant lymphomas (Braylan, Jaffe, Burbach, Berard-8; Sanders, Braylan-13); pathological staging of non-Hodgkin's lymphomas (Lotz, Berard-15); screening for breast cancer (Rabson-18); secretion of chorionic gonadotropin from cultured cells of cancers of the lung and placenta (Rabson-19); and after transplantation in the nude mouse (Tralka, Rabson-54); proglucagon-like substance in plasma of a patient with a glucagonoma (Triche-21); immunologic markers and surface characteristics of childhood lymphoblastic leukemia (Jaffe, Braylan, Berard-22); in-situ vaginal carcinoma (Henson-23); nodular hepatic hyperplasia in hemihypertrophy (Henson-24); intracytoplasmic demonstration of immunoglobulin in neoplastic cells of malignant lymphomas (Garvin-25); gonadoblastoma (Garvin-28); demonstration that Burkitt's lymphoma is a B-cell tumor (Mann, Jaffe, Braylan, Nanba, Berard-33); lymphadenopathy in subacute lupus erythematosus (Reddick-34); treatment, classification and concepts about leukemia and lymphomas (Berard, Jaffe-32; Berard-36, Berard-37; Braylan, Berard-48); study of cartilage in hemophilic arthropathy (Banfield-40); study of senescent human fibroblasts with flow microfluorometry (Fowlkes-41); herpesvirus-induced antigens in patients with squamous cell carcinoma of the head and neck (Henson-43) and in smokers (Henson-44); immunoblastic lymphadenopathy evolution into malignant lymphoma (Jaffe, Braylan-45); chickenpox, measles and mumps (Young-52); cilia formation in

cultured lung cancer cells treated with dimethyl sulfoxide (Tralka, Rabson-53); evaluation of therapy for ovarian cancer (Bunnag-58); organisms mimicking Pneumocystis carinii (Young, Chu-60); Banti's syndrome in vinyl-chloride-induced hepatic angiosarcoma (Thomas-10). Reference numbers are to the attached list of 1976 publications.

There are a large number of other on-going studies of human disease which are listed in individual annual reports. A list of some of them includes: comparative reviews of osteosarcoma (NIH and Boston Children's Hospital cases by Drs. Hanson and Costa together with Drs. Ackerman and H. Dorfman) (Stockholm cases treated with interferon reviewed by Dr. Thomas together with Dr. D. Dahlin); hepatic angiosarcoma due to Thorotrast and arsenic (Dr. Thomas, Dr. N. Telles and Dr. H. Popper in collaboration with Dr. H. Falk, epidemiologist with the CDC); review of HIP breast cancer cases (Dr. Thomas and Dr. T.A.S. Hanson together with Drs. L. Ackerman and R. McDivitt); review of some 600 NCI cases of non-Hodgkin's lymphoma (Dr. Garvin and Dr. Berard); review of all NCI soft tissue sarcomas (Drs. Costa and Webber); Wegner's granulomatosis and lymphomatoid granulomatosis cases (Drs. Costa, Webber and Berard); Waldenström's macroglobulinemia cases (Drs. Mann and Berard); thyroid lesions in patients with radiation exposure (Dr. Chu); study of myosins in soft tissue tumors (Dr. Henson); national study of pathologic evaluation of ovarian cancer (Dr. Costa - reference pathology center is under Dr. S. Gallagher, M.D. Anderson Hospital); review of 120 breast cancer cases from Tunisia (Drs. O'Conor, Costa and Webber); E.M. studies of childhood and adult soft tissue sarcomas (Triche and Reddick).

Miss Bowling and her staff continue to provide excellent histological slides and special stains for human tissues. She is also a valuable consultant on histologic techniques to numerous NIH scientists and Clinical Center physicians. The handling of human tissues is highly integrated with the performance of autopsies and the use of human tissues for special studies. The autopsy assistants, Mr. Herndon, Mr. Dock and Mr. Mattison, play a major role in assisting residents in the actual performance of the autopsies and in the fixing, distribution and storage of human specimens.

Photographic services for human tissues and experimental studies are provided by Mr. K. Edwards, Mr. Banks and Mr. Isenburg. Mr. Banks also maintains the slide files which now number over a million slides of human tissues accumulated since the Clinical Center opened in 1953. These are frequently used for comparative and retrospective studies and constitute an invaluable archive of human diseases studied at the Clinical Center. The photographic laboratory is also used by numerous Laboratory of Pathology investigators and technicians, especially by those who develop and process photomicrographs for E.M.

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Cytopathology Section

Dr. Chu and her staff provide complete diagnostic service in exfoliative cytology, medical cytogenetics and fine needle aspiration. Chromosomal analysis includes standard karyotyping and special techniques for the identification of individual chromosome pairs. During the year more than 4000 cytology specimens were accessioned. These represented over 9500 smears which were examined and reported. Approximately 1/3 of these were vaginal and cervical specimens and nearly 2/3 (2,684) were other types of specimens including ascitic, pleural, gastric, prostatic and spinal fluids, bronchial specimens, wound washings and fine needle aspirations. In addition to the human diagnostic service and studies, Dr. Chu and her staff are participating with NCI Surgery Branch staff in experimental studies of cytogenetic changes in chemically induced colonic tumors and chemically induced sarcomas produced in hamsters and rats. Dr. Chu and staff have major teaching responsibilities for the residents in the Laboratory of Pathology and for pathology residents from Georgetown University Hospital and Walter Reed who spend a few weeks or months visiting her laboratory to study general cytology and fine needle aspiration. Dr. Chu has major responsibilities for a collaborative study on cytologic screening of potential bladder cancer patients in Egypt who have Bilharzial cystitis. Mr. Pat DelVecchio has also participated in this study as a cytology and technical advisor and has visited the experimental study unit in Cairo which is under the direction of Dr. Nabil El-Bolkainy who is at the Cairo Cancer Institute.

Quantitative Cytology Section

Dr. Chester Herman and his staff including Dr. Bunnag, Dr. Alabaster, Ms. Cassidy and Ms. Fowlkes are responsible both for the intramural research and for supervision of the extramural contracts under the DCBD Cytology Automation Program. The intramural research includes several major projects. Among them is the use of cytochemical methods for analyzing the macromolecular content of cells such as use of propidium iodide-fluorescein isothiocyanate staining of gynecologic specimens and analysis of the histone content and histone fractions of cells. Dr. Joachim Betz of the Batelle Institute, Frankfurt, will work with Dr. Herman and staff from July to December, 1977 under the USA-German Exchange Program as an Expert. He will compare immunofluorescent quantitation of histone fractions with cytochemical staining of the same fractions. A second major effort is to develop analytic techniques for multiparameter flow data. The LASL sorter is now on line with the PDP 11/40 system on the fifth floor and Dr. Marvin Shapiro (DCRT) and Dr. Lance Liotta, a resident in pathology, are working with taped data to evaluate a number of algorithms for quantitive definition of normal and abnormal gynecologic cytologic materials stained with PI-FITC. Dr. Alabaster has continued work with the L1210 ascites and P388 leukemia lines treated with chemotherapeutic agents. Flow microfluorometry data have been analyzed with cytosine arabinoside, vincristine and maztansine used singly or in combination. These studies indicate a definite ability

to predict tumor cell responsiveness to a combination of agents based on life cycle analysis of cells following treatment with individual agents. Several collaborative projects are underway or completed including FMF study of Fc receptors on herpes simplex virus transformed cells (with Dr. Costa and Ms. Yee) and cell size distribution, DNA content and thymidine incorporation in hairy cell leukemia (with Drs. Braylan, Jaffe, and Berard). Two projects, one involving analysis and sorting of chromosomes and the other involving analysis of both DNA content and DNA synthetic rate in individual cells, were extensively explored with the LASL sorter. These experiments were then terminated, the former because of marginal sensitivity of the instrument and the latter because of commercial availability of better equipment and methodology.

The Image Processing Unit located in Building 37 was transferred to the Quantitative Cytology Section under Dr. Herman in March, 1977. The staff of IPU includes Dr. Lewis E. Lipkin, Medical Officer, Mr. Morton L. Schultz, Electronic Engineer, Mr. Peter F. Lemkin, Computer Specialist, Mr. Richard Gordon, Expert, Mr. Bruce A. Shapiro, Expert, Miss Marta Jo Wade, Biologist, Mr. James C. Camper, Computer Technician, and Miss Jo Olivia Abbott, Engineering Draftsman. Also Mr. Earl M. Smith joined the Unit as a Computer Scientist in an IPA position in April, 1977. Dr. Lipkin and the staff of IPU have developed computer systems for on-line measurement and characterization of both fixed and living cells. Extensive optical and image processing equipment and computer hardware have been constructed together with the development of appropriate software systems. Under Dr. Herman the IPU will continue working in computer science but will redirect their major research efforts to biological problems. Hopefully, these efforts will also involve scientific collaboration and supplementation between the on-going intramural research of the Quantitative Cytology Section using the LASL sorters and the IPU using the image processing techniques and equipment which they have developed. From the budgetary and support standpoints the IPU will be kept as a separate entity so that the efforts to redirect its work into biological research can be evaluated in the next one to two years.

Ultrastructural Pathology Section

During the year Dr. Triche, his staff and their equipment were moved from the 8th floor of the Clinical Center into completely renovated new laboratory space on the 2nd floor of the A wing. A walk-in cold room was added to his laboratory facilities and a new Phillips 400 series electron microscope was purchased. This relocation will have a major impact on the work of the ultrastructural pathology staff because of their major responsibilities for diagnostic E.M. They will now be physically located next to the surgical pathology laboratories which receive the fresh, unfixed biopsies and surgically resected specimens, cytology specimens and to the human tissues available in the autopsy room. Dr. Robert Reddick will finish residency training in July, 1977 and will join Dr. Triche as a Staff Fellow and continue to spend a major portion of his time on diagnostic E.M. During the past year, 139 human cases were received and processed for

E.M. Of these 110 were non-lymphoma cases and 29 were lymphomas. Of these cases, approximately 70-75 were completely processed and examined and in a number of instances, the E.M. findings were available within 24 to 48 hours after the specimen was received. Dr. Triche has concentrated his diagnostic E.M. studies on childhood tumors, neuroblastoma, Ewing's sarcoma and rhabdomyosarcoma/undifferentiated sarcoma, as a part of his close working relationships with the Pediatric Oncology Branch staff. Of particular interest are his observations about a case of neuroblastoma which contained glycogen. This had led to an initial incorrect diagnosis of Ewing's sarcoma. Dr. Reddick has concentrated his efforts on cases of osteosarcoma and adult soft tissue sarcomas. Dr. Triche has continued several collaborative research projects which include studies of dendritic reticulum cells in non-Hodgkin's lymphomas (with Drs. S. Watanabe, Jaffe, Braylan and Berard), virus release from murine leukemia cells following interferon inhibition (with Dr. Friedman), structural and functional characteristics of "hairy" cell leukemia (Drs. Braylan, Nanba, Jaffe, et al) and E.M. autoradiography of cell surface bound IgE on rat basophil leukemia cells (with Dr. H. Metzger).

Dr. David Zopf and Dr. A.J. Garvin will join the Ultrastructural Pathology Section staff in July, 1977. Their laboratory space will be in two modules on the 2nd floor of the A wing which were available because of Dr. Stanton's move to the 8th floor to occupy the laboratory space vacated by Dr. Triche. Dr. Zopf and Dr. Garvin have independent research programs but these will involve E.M. and they will participate in various phases of research and diagnosis of human tissues.

Dr. Henson moved his research module to the 8th floor and his office to the data processing module on the 1st floor of the A wing. He completed and published studies on thyroglobulin in human orbital skeletal muscle and has continued studies on myosins in soft tissue tumors. A major portion of his time during the year was spent with committees which developed and published two coded nomenclatures, the Systematized Nomenclature of Medicine (SNOMed, College of American Pathologists) and the International Classification of Diseases for Oncology (ICD-O, World Health Organization). He was also active on the Pan American Health Organization committees to develop and publish Spanish and Portuguese language editions of ICD-O which will be used in tumor registries in Latin America.

Hematopathology Section

Drs. Berard, Jaffe, Braylan, and their co-workers Drs. Nanba, Ricard, and Mann have major programs in diagnostic and experimental studies on neoplastic and non-neoplastic lesions of the lymphoreticular and hematopoietic systems. Dr. Berard has an enormous referral of cases of malignant lymphomas including 165 cases from the Eastern Cooperative Oncology Group submitted to him as Regional Pathologist for the Pathology Panel for Lymphoma Clinical Trials and 516 cases submitted for diagnostic consultation from pathologists in the United States and foreign countries. In addition, he and his staff are responsible for the diagnostic reports on cases of malignant lymphoma admitted to various clinical services in the Clinical Center. Fresh biopsy

tissues were usually available for the Clinical Center cases and during the past year more than 80 such specimens were used by Drs. Jaffe, Braylan, Nanba and Mann for special immunologic, histochemical and ultrastructural studies. This is a very productive program as indicated by the relatively large number of publications (see attached list of publications). Included were studies on the immunologic, histochemical and morphologic features of cases of macroglobulinemia of Waldenström, non-endemic ("American") Burkitt's tumor, "hairy" cell leukemia, Sezary's syndrome, childhood lymphoblastic lymphoma, immunoblastic lymphadenopathy, and a variety of other malignant lymphomas and leukemias. Of particular interest, were the new observations about splenic pseudosinuses and hepatic angiomatous lesions which are distinctive pathologic lesions of "hairy" cell leukemia and which were published with Dr. Nanba, Mr. Soban and Miss Bowling.

Drs. Berard, Jaffe and Braylan were each invited to give several lectures at scientific meetings and continued to serve as faculty members for the Tutorial on Neoplastic Hematology organized by Dr. H. Rappaport at the University of Chicago. These educational efforts have been ongoing for several years and briefly mentioned in previous annual reports but their importance has not been specifically stressed. The developments in the diagnosis and classification of the malignant lymphomas have been particularly dynamic during the past several years because of rapidly increasing knowledge about the functional characteristics of the neoplastic and normal cells of the lymphoreticular and hematopoietic systems. Dr. Berard, Dr. Jaffe, Dr. Braylan and their associates have made major contributions to this increased knowledge and also have participated in many national and international meetings, symposia and tutorials to bring this new information to practicing, diagnostic pathologists. They have served as faculty members of the 9th Tutorial on Neoplastic Hematopathology conducted at the University of Chicago and the 2nd International Tutorial held in Geneva, Switzerland in August, 1976. Dr. Berard gave principal lectures at the University of Virginia Medical School, Suffolk County (New York) Society of Pathologists, Leukemia Society of Massachusetts, University of Minnesota Medical School, Massachusetts General Hospital, and American Society of Hematology. He was chairman of the pathology session of a Symposium on Non-Hodgkin's Lymphomas in San Francisco in September, 1976 and presented the Annual Slide Seminar of the New Jersey Society of Pathologists in November, 1976. Dr. Braylan gave lectures at the University of Cologne, at the American Society of Hematology meeting in Boston in December, 1976 and was a participant in meetings of the WHO/International Union of Immunological Societies meeting in Munich in October, 1976, and the Fifth National Congress of Pathology in Lima, Peru in November, 1976. During the past year, Dr. Jaffe gave the Third Annual Ruth M. French lecture at the University of Illinois and lectured at the University of Chicago, American Society of Hematology meeting, at the IAP meeting in Toronto, Canada, the Washington Hospital Center and at the San Francisco Symposium on Non-Hodgkin's Lymphomas. This sample of their recent lectures is cited in order to stress the importance of their teaching efforts nationally and internationally and the impact this has had for diagnostic pathologists in hematopathology.

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Drs. Berard, Braylan, Jaffe, and Mann participate fully in other activities of diagnostic pathology including the teaching of residents and are responsible for several clinico-pathologic conferences. In addition, Dr. Jaffe is chairman of the departmental residency selection program.

Comparative Oncology Section

Drs. Dawe, Banfield, and Stanton are the professional staff members of this Section. Dr. Dawe has continued his studies of epithelial-mesenchymal interactions during the development of neoplasia. During the past year he has demonstrated that neoplastic transformation of polyoma virus infected epithelial cells cultured on millipore filters can be induced in the absence of mesenchyme. Four transformed lines are in continuous culture and produce tumors when transplanted into syngeneic mice. A major finding from these studies is that the tumor lines (induced in the absence of mesenchyme) are different from those induced in the same cell type in the presence of salivary gland mesenchyme in that they are more highly invasive and frequently metastasize. Also these undifferentiated tumors occasionally show differentiation as squamous cell carcinomas or as adenocarcinomas. This experimental model will now be used to attempt to define factors which direct these types of differentiation. Dr. Orlando, working with Dr. Dawe, is studying polyoma virus-induced mammary tumors in athymic nude mice. Study of the smallest of these tumors indicates they do not originate from the hyperplastic alveolar nodules that usually give rise to spontaneous and MTV-related mammary tumors in mice. Dr. Sakakura worked with Dr. Dawe 2 or 3 years ago on mesenchymal control of mammary gland morphogenesis and differentiation. Part of this work was completed after she returned to the Aichi Cancer Institute in Nagoya, Japan, and has been published. In experiments which she has continued, she has shown that mammary epithelium recombined with salivary mesenchyme undergoes morphogenesis characteristic of salivary gland. However, under the influence of pregnancy in hosts of these heterotopic recombinations, the "hybrid" organs secrete the B protein (α-lactalbumin) characteristic of milk. This experimental model also will be used for additional studies on control of differentiation and function. Mr. Willie Morgan, in time-lapse motion picture studies of normal mouse salivary gland epithelium in organ culture, has discovered periodic contractile movements in epithelial rudiments. Several types of contractile movements, some synchronous in different parts of the rudiments, have been observed and are being studied for correlations with myoepithelial cell growth in the rudiments. Dr. Kurokawa, an NIH Fellow, working with Dr. Dawe is studying the relationship of alterations in gastric stroma to the response of gastric epithelium to chemical carcinogens. In these experiments he has combined gastric and intestinal stroma with gastric and intestinal epithelium, exposed these various tissue components to chemical carcinogens and transplanted them to syngeneic hosts. These experiments are in progress and animals are just now being autopsied but one animal with ENNG-exposed forestomach epithelium recombined with forestomach mesenchyme has been scarificed and a small undifferentiated carcinoma was found at the transplantation site.

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Dr. Stanton has continued his studies of pulmonary carcinogenesis with polynuclear hydrocarbons and tobacco products. In these experiments he is studying the influence of various local factors including nitrates, vitamin A analogs and polynuclear inhibitors on tobacco-induced pulmonary cancers. His major studies on the tumorgenicity of various fibrous materials and fiber sizes are being continued. These include fibrous glass, potassium titanate, aluminum oxide, dawsonite, silicon carbide, gypsum and talc. His studies indicate that large diametered fibers and minute short fibers are adequately handled by phagocytic activity, the latter by phagocytosis, the former by a process of fusion and plating of the surface of the particulate material. However, fibers that have diameters <1.5 μm and lengths greater than 8 μm are not handled by phagocytes but initiate collagen deposition and result in a high incidence of pleural neoplasms. Dr. Stanton, in collaboration with Dr. Banfield using electron probe analysis, plans to study various pulmonary dusts and foreign bodies in other tissues and organs. Dr. Stanton will move his experimental laboratory to the 8th floor during the summer of 1977. As a part of his experimental work, Dr. Stanton is project officer for a contract with Pharmacopathics, Inc. which provides him with long-term animal holding facilities.

Dr. Banfield, during 1976, published his E.M. observations on the cylindroid lamella-complexes and nucleoid intracytoplasmic bodies in lymphoma cells of the northern pike. This was work performed in collaboration with Drs. Dawe, Sonstegard and Mr. Lee and Mr. Michelitch. He is currently studying a similar lamella-particle complex observed in the nuclei of owl monkey kidney cells infected with Herpesvirus saimiri and also similar structures ("Kolmers crystalloids") found in horizontal cells of the human retina. As noted above he plans to collaborate with Dr. Stanton in electron probe studies of various types of pulmonary dust and particulate material.

Viral Oncology and Molecular Pathology Section

Dr. N.A. Young has continued experimental studies on the structure and assembly of vaccine polioviruses. He previously had shown that structural instability of the largest virion polypeptide, VP1, of type 1 Sabin poliovirus results from a modification of a precursor polypeptide occurring before assembly into the procapsid. During the past year, he has shown that the increased neurovirulence of poliovirus strains recovered from the feces of children fed oral vaccine is due to back-mutation of the virus to manufacture structurally normal, stable VP1 polypeptide. "Antigenic drift" is also explained by these observations. They also probably explain the relatively high incidence of occurrence of vaccine-associated paralytic poliomyelitis in immunodeficient children. The hypothesis is that the lack of neutralizing antibodies to the vaccine strain in the immunodeficient patient contributes to the mutant virus emerging as the predominant strain in the gut. Dr. Young, with Ms. Engel and Ms. Tralka has continued studies of cultured lines of human breast cancer. One observation of interest about these culture lines is that 2 lines from one

patient have different biological and hormonal properties. Each has different chromosome abnormalities and the 1st line is estrogen responsive while the 2nd line appears to be testosterone and glucocorticoid responsive. The patient had been treated with anti-estrogens between the time the 1st and 2nd culture lines were established. Attempts to detect or recover oncornavirus from these 3 human breast cancer lines have been negative. Further studies to detect subviral expression of oncornaviruses in these cell lines are being made. Dr. Strauchen has continued his studies on the effect of interferon on mouse mammary tumor virus expression. His experiments suggest that the effect of interferon is to block MMTV production in a late stage of assembly or release from the cell membrane. Dr. Young, with Mrs. Keith, have continued to culture viruses from autopsy tissues. Among the immunosuppressed patients, the most commonly cultured viruses are herpes simplex, cytomegalovirus and varicella-zoster. No isolates of myxoviruses or enteroviruses have been made. Thus it seems unlikely that respiratory viruses other than herpesviruses play a significant role in pneumonias of the compromised host.

Ms. Tralka and Mr. Henson, in the E.M. lab of VOMP processed over 1600 tissue blocks and examined approximately 300 specimens by E.M. Over 3000 photographs were taken and approximately 4000 micrographs were printed. They collaborated in a number of studies with other scientists including E.M. study of a cultured line of thyroid tissue which produces prostaglandins (Dr. Zusman), synthesis of prostaglandins in toad bladder epithelium after treatment with arachidonic acid (Dr. Zusman), tissue culture lines in the presence of differentiating agents such as DMSO (Dr. Rabson), a granulosa-SV40 transformed cell line (Dr. Rabson), effect of sodium butyrate on human lung carcinoma cells (Dr. Rosen), nude mouse mammary tumor cell line developed from polyoma virus (Dr. Dawe), morphogenesis of 2 human papovaviruses in human embryonic cells (Dr. Costa), and an undifferentiated lymphoma cell line treated with theophyllin (Dr. Jaffe).

Dr. Costa, in addition to his work as Head of the Surgical Pathology and Postmortem Section, continues an active research program in the VOMP Section. Much of his work is in collaboration with Dr. Rabson. Included are studies on the morphology of H. saimiri in two cell lines, the characteristics of MMV particles replicating in HEK cells have been analyzed by CsCl banding and correlated to the morphology of replication, the effect of tetrameric concanavalin A and succinyl concanavalin A on H. simplex virus, the effect of IgG and Fc fragments of IgG on H. simplex infected cells and the effect of bisacetamides on a variety of cell lines. Among the latter experiments an observation of particular interest is that bisacetamides stimulate collagen synthesis in a cell line derived from human glioblastoma multiforme. Dr. Robert Stern is participating in this study.

In addition to completing residency training, Dr. Peter Howley is continuing his studies on the genomes of human papovaviruses, BK and JC, and of the mouse papovavirus, K, and testing the ability of portions of the BK viral genome to transform cultured human cells. He is also examining human

tumors for the presence of human papovavirus. During the year he completed experiments showing that a viral isolate from Macaques with progressive multifocal leukoencephalopathy was SV40 virus. This work, and several other on-going experiments cited above, are in collaboration with Dr. K. Takemoto.

Pathological Technology Section

Part of the technology staff, under Mrs. Barbara Coolidge, is located in Building 37 and part of the staff, under Mrs. Ruby Howard, is in Frederick, Maryland. They provide histological sections and special stains for animal tissues for scientists in the DCBD. During the year they processed more than 31,000 blocks of tissue, prepared over 41,000 H & E slides and more than 3,000 special stains. There were a total of 108 NCI investigators who submitted material for sectioning. Also during the year 154 blocks of tissue were embedded for E.M. Of these, sections were prepared on approximately 140. 208 photomicrographs were taken and nearly 500 pictures were printed. The quality and performance of this section's staff continues to be outstanding and they represent a major technical resource for the research of scientists in the NCI.

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Publications-1976

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Publications-1976

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Publications-1976

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00853-24 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Surgical Pathology and Postmortem Section

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J.C. Costa Medical Officer LP NCI
(see next page for additional staff)

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Surgical Pathology and Postmortem Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
20	20	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgical Pathology and Postmortem Section, together with the Cytopathology Section, Ultrastructural Pathology Section and Hematopathology Section provide complete service in anatomic pathology for the Clinical Center patients and collaborate with the research staff of all Institutes in those investigations which involve the use and study of human pathological material. The Staff of the Surgical Pathology and Postmortem Section also carries on independent and collaborative research.

PHS-6040
(Rev. 10-76)

Project Description:

Staff:

Dr. Louis B. Thomas, Chief, Laboratory of Pathology
Dr. José C. Costa, Head, Surgical Pathology and Postmortem Section
Dr. Bruce L. Webber
Dr. Donald E. Henson
Dr. Chester J. Herman, Head, Quantitative Cytology Section
Dr. Bill Bunnag
Dr. Oliver Alabaster
Dr. Costan W. Berard, Head, Hematopathology Section
Dr. Raul C. Braylan
Dr. Elaine S. Jaffe
Dr. Elizabeth W. Chu, Head, Cytopathology Section
Dr. Timothy J. Triche, Head, Ultrastructural Pathology Section
Dr. Clyde J. Dawe, Head, Comparative Oncology Section
Dr. William G. Banfield
Dr. Mearl F. Stanton
Dr. Nathaniel A. Young, Head, Viral Oncology and Molecular Pathology Section
+Dr. Peter M. Howley
+Dr. James A. Strauchen
+Dr. Robert L. Reddick
+Dr. Lance A. Liotta
+Dr. William J. Cook
+Dr. Jan M. Orenstein
*Dr. Allen W. Cheever, National Institute of Allergy and Infectious Diseases
*Dr. Robert M. Friedman, National Institute of Arthritis, Metabolism, and Digestive Diseases
*Dr. William C. Roberts, Chief, Laboratory of Pathology, National Heart, Lung, and Blood Institute
*Dr. Marius Valsamis, Associate Professor of Pathology, New York Medical College, Center for Aging and Chronic Diseases

The objectives of the Surgical Pathology and Postmortem Section are: (1) to provide diagnostic services in pathologic anatomy; (2) to collaborate with National Institutes of Health investigators in research involving use and study of human material; (3) to conduct a residency training program in pathologic anatomy, and (4) to carry out independent research.

+These physicians are full time Residents in the Laboratory of Pathology
*These Associate Pathologists spend part time in the activities of the Surgical Pathology and Postmortem Section.

1. The service functions of the section during the past year included:

(a) 311 autopsy examinations. The residents perform nearly all of the postmortem dissections under supervision of the staff. A gross review is held by the staff and residents each morning at 8:30. The residents review the microscopic slides of each autopsy with one of the staff before completing the autopsy protocol.

The staff assists the residents in preparing for the numerous clinical conferences in which the Section participates.

(b) 8960 specimens were accessioned in the Surgical Pathology and Postmortem Section. These specimens are initially examined and described by a resident and their reports are checked by the staff. Associate pathologists from other laboratories or sections are frequently consulted about problems in diagnosis in surgical pathology and/or assume responsibility for handling certain tissue removed for specific research projects.

(c) Histological preparation and special procedures:

These functions are carried out under the direction of Miss Mary C. Bowling and a complete listing of specimens processed is given in the accompanying table.

(d) Photographic services of the department:

A photographic record is made of the large amount of the pathological material which is handled and studied in the department. The photography is done by the staff and residents with the technical assistance of Mr. L. Kenzie Edwards who also maintains the equipment and carried out much of the photographic processing. Gross photographs and photomicrographs of the pathologic material are provided to the clinical staff on request, and are used extensively for conferences, seminars and many are prepared for publication. Listed below is a breakdown of the work performed by Mr. Edwards from April 1976 through March 1977.

Time Taking Photographs:	475 hours, 55 minutes
Time Processing:	261 hours, 45 minutes

No. of Black and White Negatives: 4 x 5 inches	2,504
Color Transparencies - 4 x 5 inches - gross:	1,230
35 mm. Transparencies - gross:	9,906
Total	11,136
Prints Contract Out - 4 x 5 inches - black and white:	7,932

A large proportion of the photomicrography is done under the direction of Mr. Ralph Isenburg, who provides professional assistance and facilities for the entire staff of the Laboratory of Pathology.

2. The collaborative activities of the staff and residents in the Surgical Pathology and Postmortem Section are reflected in the summary of research activities listed below:

(a) Distribution of human organs and tissues:

At present there are approximately 20 scientists in the various laboratories at the National Institutes of Health who have requested particular tissues from surgical and postmortem services. On many occasions it is possible to furnish these investigators with fresh human material. A list of these investigators is kept by the secretarial staff and telephone calls are made to the various investigators whenever surgical or postmortem tissues are available.

(b) Conferences:

The staff and residents take an active part in numerous Clinical Center and Institute conferences. In addition, there are several conferences regularly organized solely or jointly by the department, including a Surgical Pathology Conference, Joint Pathologists Conference, Neuropathology Conference, Lymphoma Staging Conference, Medical Breast Cancer Service Pathology Conference, Medicine Branch Slide Review Conference, Pediatric Oncology Conference, Radiation Therapy Conference, Gastrointestinal Disease Conference, Brain Cutting Conference and Gross Autopsy Conference.

Proposed Course: (1) Continue to provide the services described; (2) Increase the interaction with the clinical branches in the design and evaluation of protocols; (3) Improve the opportunities for the resident staff to participate in teaching, conferences, and seminars, and provide elective periods to be spent accomplishing research projects with the senior staff; (4) Implement data retrieval programs.

LABORATORY OF PATHOLOGY, NCI
SURGICAL PATHOLOGY AND POSTMORTEM SECTION
February 1976 through April 1977

SURGICAL PATHOLOGY	
Specimens accessioned	8960
Blocks of tissue cut	17524
Blocks of tissue recut	1329
Slides made	60782
Slides stained H&E	41370
Slides stained special	8085
Frozen sections	612
POSTMORTEM PATHOLOGY	
Autopsies performed	213
Autopsies submitted	98
Blocks of tissue cut	10846
Blocks of tissue recut	849
Slides made	16817
Slides stained H&E	12661
Slides stained special	1891
COMBINED TOTAL	
Total blocks cut	30548
Total slides made	77599
Total H&E	54031
Total special stains	9976

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CB 00852-24 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Exfoliative Cytology Applied to Human Diagnostic Problems and Research Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.W. Chu	Medical Officer	LP NCI
OTHER:	P.R. DelVecchio	Super. Res. Biologist	LP NCI
	S.D. Byrne	Res. Biologist (Cytol.)	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Cytopathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Provides complete diagnostic service in exfoliative cytology, medical cytogenetics, and fine needle aspiration cytology. In addition, collaborates in various clinical research projects utilizing special techniques including special staining and tissue culture techniques.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1. To provide the staff of the Clinical Center with an accurate and complete exfoliative cytology service as well as diagnostic cytogenetics (karyotyping).

2. To collaborate in various clinical research projects evaluating cancer therapy, the hormonal status of the cancer patients, the course and natural history of the cancer lesions and the anatomical and physiological changes in the human body associated with various pathologic conditions.

3. Development of better diagnostic techniques.

The specific objectives include:

A) Cytology

1. Continued improvement in cytologic techniques in establishing definitive diagnosis.
2. Improvement in fine needle aspiration cytologic technique.
3. Establishing cytologic characteristics of cell types seen only in fine needle aspirations.
4. Setting up special stain techniques which may be used for general cytology or special research problems.

B) Diagnostic Cytogenetics

1. Improvement in short term culture system to assure of successful harvesting of in-vitro cells.
2. Improvement in staining techniques.
3. Improvement on special techniques such as Giemsa bandings of chromosomes and sister chromatid exchange methods.

Methods Employed:

1. Special procedures in addition to the standard Papanicolaou stain are:

A) Millipore filtration of all fluids
B) Special stains:

1. Feulgan Reaction for sex chromatin bodies
2. Acid fast, Pneumocystis, Methenamine silver, Brown Brenn for various organisms and fungi
3. PAS, Wright Giemsa, iron stain, melanin stain, congo-red, oil-red-O, mucicarmine for various specific reactions

2. Short term cultures of peripheral blood, bone marrow cells and tumor cells found in body fluids are the standard diagnostic methods.

In addition, special techniques involving trypsin digestion followed by G-Banding (Giemsa bands) are routinely offered.

Biopsy materials of skin, gonads, tumors are cultivated by tissue culture technique and cells harvested for chromosomal analysis.

Patient Material: The figures compiled below are for the last 12 months.

	March 1976 - February 1977	
Type of Material Examined:	Accessions	Smears
Vaginal and Cervical	1,120	1,908
Other (fluids, special procedures, special stains and chromosomal analysis)	2,803	7,475
Total	3,923	9,383

Sources of Material Received

Institutes	Accessions	Smears
NCI, NHLI, NIAID, NIAMDD, NIMH, NICHHD, NEI, NINCDS, EHS, NIDR and Referral	3,923	9,383
Total	3,923	9,383

Major Findings: Major contributions of the Cytopathology Section:

1. Providing prompt services and early diagnosis in cytological materials.
2. Providing cytologic evaluation and estimated ranges of either relapse or remission in the continual management of meningeal leukemia.
3. Providing cytological evaluation of therapeutic effects on urinary specimens.
4. Providing chromosomal analysis for clinical studies as well as in the establishment of definitive malignancies.
5. Providing cytologic methods to research projects specifically, fine needle aspiration in thyroid nodules and bone lesions.

Significance: 1. The diagnostic value of exfoliative cytology in the clinical management of various disease states is an established fact.

2. Aspiration cytologic techniques are useful in establishing diagnosis on metastatic diseases and/or recurrent malignancies. In such instances, the fine needle aspiration technique eliminates the more involved incisional biopsy and therefore is more economical to the clinicians as well as to the patients.

3. Chromosomal analysis is a way of establishing definitive diagnoses in selected patients with endocrine disorders, congenital defects and also in some malignancies.

Proposed Course: 1. Continue to provide a complete cytodiagnostic service for the entire staff of the Clinical Center in various research projects.

2. Continual improvement and development of established as well as new techniques in cytologic and cytogenetic fields.

Activities: From July 1, 1976 to September 1977

1. Presented work on Thyroid Cytology, conference on Thyroid Cancer, University of Chicago, October 30, 1976.

2. Lecture on General Cytology, Building 10, Room 4B36, January 3, 1977.

3. Lecture and workshop on Fine Needle Aspiration of Breast Lesions; Tutorial in Clinical Cytology, Chicago, April 2, 1977.

4. Lecture and workshop on Fine Needle Aspiration Cytology, Illinois Society of Cytology, April 22-23, 1977, Chicago, Illinois.

5. Panelist on Breast Cytology, American Society for Medical Technologists, Atlanta, Georgia, June 20, 1977.

Publications:

1. Gershengorn, M.C., McClung, M.R., Chu, E.W., Hanson, T.A.S., Weintraub, B.D., and Robbins, J.: Fine needle aspiration cytology in the pre-operative diagnosis of thyroid nodules. Ann. Intern. Med. (in press).

2. Tralka, T.S., Costa, J.C., Sindelar, W.F., Rabson, A.S., Gullino, P.M., Henson, E., Chu, E.W., and O'Conor, G.T.: Spontaneous lymphosarcoma arising in a nude mouse: Characterization in vivo and in vitro. J. Natl. Cancer Inst. (in press).

3. Young, R.C., Bennett, J.E., and Chu, E.W.: Organisms mimicking Pneumocystis carinii. Lancet II: No. 7994, 1082-1083, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00582-06 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chronic Mouse Cytomegalovirus Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.E. Henson	Medical Officer	LP NCI
OTHER:	R.E. Levinson	Biologist	LP NCI
	R.D. Smith	Medical Officer	Univ. of Cincinnati

COOPERATING UNITS (if any)

University of Cincinnati School of Medicine

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the possible reactivation of chronic mouse cytomegalovirus infection by immunosuppression and anti-lymphocytic serum (ALS). Administration of ALS shortly after acute infection leads to a chronic disseminated disease with glomerulonephritis.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Study the mechanisms of chronic murine cytomegalovirus infection in the salivary glands and lymphoid tissue of mice chronically infected with this virus and effect of anti-lymphocyte serum on infection.

Methods Employed: Tissue culture, immunofluorescence, electron microscopy.

Significance: The mouse cytomegalovirus causes chronic infection in the submaxillary glands, lymph nodes, and spleens of many strains of mice. Infection in the lymph nodes and spleen probably occurs in lymphocytes. The murine cytomegalovirus belongs to the herpesvirus group of animal viruses, along with the Epstein-Barr virus and Herpesvirus saimiri.

Proposed Course: Part of this study is completed. Results indicate that chronic CMV infection does not enhance or accelerate natural development of lymphoma in mice. Present studies involve observations on the course of acute and chronic infection following the administration of anti-lymphocytic serum.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00585-04 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Histological Evolution of In-Situ Carcinoma in the Vagina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.E. Henson	Medical Officer	LP NCI
OTHER:	G.W. Geelhoed	Medical Officer	Dept. Surg., G.W. Univ.

COOPERATING UNITS (if any)

George Washington University School of Medicine

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to review biopsy material from the vagina of patients who have had a previous carcinoma of the cervix in order to evaluate the vaginal epithelium for early in-situ changes.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Clinical-pathological study of women with multicentric squamous cell cancers in the ectogenital epithelium and relationship of in-situ vaginal cancer to subsequent invasive cancer.

Major Findings: Histological studies made on women with sequential vaginal biopsies have demonstrated progression from severe epithelial atypia, through dysplasia, to carcinoma in-situ. Like carcinoma of the cervix, in-situ vaginal tumors have an invasive potential. In-situ vaginal tumors associated with carcinoma of the cervix seem to form a distinctive clinical syndrome.

Significance: Many women with cancer of the uterine cervix subsequently develop epithelial cancers in the vagina and/or vulva. These multiple cancers have been considered to arise in an epithelial region predisposed to malignancy. This region has a common cloacal origin embryologically.

Proposed Course: Project is still in effect. Patients will be added to the study as they are diagnosed. A report has been published.

Publications:

1. Geelhoed, G., Henson, D., Taylor, P., and Ketcham, A.: In situ vaginal carcinoma of the cervix. A distinctive clinical entity. Am. J. Obstet. & Gynecol. 124: 510-516, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00591-03 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunofluorescence Studies on Human Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.E. Henson	Medical Officer	LP NCI
OTHER:	R.E. Levinson	Biologist	LP NCI
	R.S. Adelstein	Medical Officer	IR CB NHLI

COOPERATING UNITS (if any)

IR CB NHLI

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range of this project to study the differences of the myosins and other proteins present in normal tissue, rhabdomyosarcomas, osteogenic sarcomas, and other soft tissue tumors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Use of immunofluorescence on frozen section material to look for novel or unusual proteins synthesized by human tumors.

Methods Employed: Immunofluorescence immunology.

Major Findings: Dedifferentiated rhabdomyosarcoma cells produce a new type of myosin immunologically and biochemically related to myosin present in fibroblasts. Myosin might be helpful in diagnosis of rhabdomyosarcomas.

Significance: The purpose is to search for novel proteins in tumors and to determine if these proteins can be useful in the early diagnosis of cancer, diagnosis of precancerous lesions, and for predicting prognosis.

Proposed Course: Test a variety of tumors by immunofluorescence using antibodies prepared against purified proteins that have been isolated from tumors, fetal tissue, normal organs, or from other sources.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00592-03 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Morphologic Investigation of Virus Induced Membrane Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.J. Triche	Medical Officer	LP NCI
OTHER:	J.C. Costa	Medical Officer	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project attempts to elucidate the relationship between membrane antigens and an Fc receptor which appear in Herpes simplex type II-infected cultured human retinoblastoma cells. Further, both of these will be related to the increased Con A lectin binding ability of HSV II-transformed cells previously noted in the literature. The appearance of an Fc receptor and increased lectin binding of a following infection time when viral-directed membrane antigens are appearing suggests a relationship between these three membrane events. We suspect the Fc receptor is a viral-directed membrane-associated protein, as are the increased Con A binding sites. We are attempting to prove or disprove this assumption using ferritin and hemocyanin-labelled antibodies and lectins. The ability to bind host IgG may confer survival advantages to HSV-infected cells, enhancing both viral replication and latency.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1. To characterize the membrane antigen (MA) of herpes simplex with regard to the time of appearance following infection, the distribution, and the association with lectin receptor sites, in cultured retinoblastoma cells.

2. To compare these results with those in the literature, and to determine the association, if any, of these membrane sites with the increased agglutinability of transformed cells by the lectin concanavalin A.

3. To compare the MA sites with the Fc receptor which also appears following HSV infection.

Methods Employed: 1. Freeze etch electron microscopy, utilizing high resolution platinum-carbon replicas of fractured and etched cell surfaces examined in the transmission electron microscope.

2. Labelling of these surfaces with ferritin or hemocyanin-tagged lectin or immunoglobulin.

Significance: 1. The known increased agglutinability of transformed cells by plant lectins (such as concanavalin A) may be related to an alteration in number or distribution of cell surface receptors for lectins, which may coincide with the appearance of viral-associated material in the membrane.

2. The HSV-directed Fc receptor may play a role in virus latency and oncogenesis.

3. A double label study, labelling both viral antigen-associated membrane sites and lectin binding sites, should provide unequivocal evidence, pro or con, of the means whereby viruses induce increased cell agglutinability, a property of cells associated with oncogenesis.

Proposed Course: Considerable time will be spent preparing reagents for labelling the membranes as well as installing the freeze etch device. Once the technological aspects are assimilated, the project will progress forthrightly through preliminary experiments and finally through repetitive testing to establish reproducibility. Later, other parameters, such as cell type, virus type, temperature, type of lectin, etc. will be evaluated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00594-1 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pathology of Family Cancer Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.E. Henson	Medical Officer	LP NCI
OTHER:	D. Parry	Medical Officer	CEB NCI
	J.J. Mulvihill	Medical Officer	CEB NCI
	J.F. Fraumeni, Jr.	Medical Officer	EEB NCI
	W.A. Blattner	Medical Officer	EEB NCI

COOPERATING UNITS (if any)

Epidemiology Branch, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Ultrastructural Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ¹

Review histopathology material from families with high incidence of cancer. These families are being followed by the Epidemiology Branch.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Review and confirm the histological diagnoses on families that have a high incidence of cancer and which are being followed by the Epidemiology Branch.

Methods Employed: Light microscopy, special histochemistry stains.

Significance: Confirm diagnoses of cancer and apply uniform terminology and classification to the lesions so that patterns may be found.

Proposed Course: Continue to review surgical pathology or autopsy material submitted from referring hospitals and report results to the Epidemiology Branch.

Publications:

1. Everson, R.B., Museles, M., Henson, D.E., and Grundy, G.W.: Focal nodular hyperplasia of the liver in a child with hemihypertrophy. J. Pediatr. 88: 985-987, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00504-21 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

An Electron Optical Investigation of Normal and Pathologic Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.G. Banfield	Medical Officer	LP NCI
OTHER:	J. Peng	Medical Officer	MO NCI
	C.J. Dawe	Medical Officer	LP NCI
	C.W. Lee	Biologist	LP NCI
	E. Lee	Biologist	MO NCI
	T.S. Tralka	Biologist	LP NCI
	A.S. Rabson	Medical Officer	LP NCI
	W.D. Morgan	Biologist	LP NCI

COOPERATING UNITS (if any)

Cytogenetic Oncology Section, Medicine Branch (Dr. Peng)

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	4	4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The transmission electron microscope is applied to significant problems directly or periferally related to neoplasia. The major effort has been a study of a cylindroid lamella-particle complex in the cells of the malignant lymphoma of northern pike and its relation to a similar structure in human "hairy cell" leukemia and other human lymphomas and leukemias and in tissue culture cells infected with Herpesvirus saimiri.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1. To describe and interpret the ultrastructure of selected pathologic states and to compare the results with suitable controls.

2. To look for and study the inciting agent or accompanying agent in neoplastic and other disease states using human, animal and tissue culture material.

Major Findings: Two papers on the lymphoma of the northern pike were published.

Summary--Cells of the malignant lymphoma of northern pike of Canada contain a cylindrical structure with a central core of cytoplasmic elements and walls of lamellae in a spiraled arrangement separated by ribosome-like particles or membranes. These cylindroids are similar, if not identical to structures in the cells of certain human lymphomas and leukemias. In cross and longitudinal section the lamellae showed a pattern of light and dark bands resulting in a 95°A period which has not as yet been described in the human cylindroids. The pike lymphoma is the only presently known animal tumor affording opportunity to investigate these interesting structures.

A similar complex occurring in stacked, tubular and twisted ribbon-like configurations was described in the nuclei of confluent monolayer cultures of primary owl monkey kidney cells infected with Herpesvirus saimiri.

Significance: A study of the cylindroid structure in the transmissible pike lymphoma when correlated with the studies on the similar structure found in human "hairy cell" leukemia may lead to a better understanding of the latter. The finding of lamella-particle complexes associated with Herpesvirus infection, a virus that can produce leukemia in appropriate hosts, suggests the possibility that viruses may also be associated with lamella-particle complexes seen in some human leukemia cells.

Proposed Course: A major effort will be made in collaboration with Dr. Jacqueline Peng to isolate and to characterize by electron microscopy a hematopoietic stem cell in human peripheral blood (Science 190: 284, 1975).

Publications:

1. Dawe, C.J., Banfield, W.G., Sonstegard, R., Lee, C.W., and Michelitch, H.J.: Cylindroid lamella-particle complexes and nucleoid intracytoplasmic bodies in lymphoma cells of northern pike (Esox lucius). In Homburger, F. (Ed.): Progress in Experimental Tumor Research. Basel, S. Karger, 1976, vol. 20, pp. 166-180.

2. Banfield, W.G., Dawe, C.J., Lee, C.W., and Sonstegard, R.: Cylindroid lamella-particle complexes in lymphoma cells of northern pike (Esox lucius). J. Natl. Cancer Inst. 57: 415-420, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00505-07 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pulmonary Carcinogenesis: Testing and Examining the Mechanisms Through Animal Models

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.F. Stanton	Medical Officer	LP NCI
OTHER:	G. Akagi	Guest Worker	LP NCI
	A. Tegeris	M.D.	Pharmacopathics, Inc.
	O.T. Chorty	Ph.D.	U.S. Dept. of Agriculture
	C. Kommineri	D.V.M.	Environ. Protec. Agency
	E. Kent	Biologist	Pharmacopathics Inc.
	E. Miller	Biologist	LP NCI
	M. May	Biologist	LP NCI

COOPERATING UNITS (if any) Johns-Manville Res. & Engineering Center, Manville, N.J.; Owens-Corning Fiberglass Corp., Toledo, Ohio; Roswell Park Mem. Center for Cancer Research; Pharmacopathics Inc., Richard B. Russell Agricult. Res. Center, USDA, Athens, Ga., Nat. Environ. Res. Center, EPA, North Carolina

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This program is designed to investigate the major causes and mechanisms of pulmonary cancer in man through the study of lung cancer induced in animals. We are studying the carcinogenic potential of subfractions of air pollutents and cigarette smoke condensate with our system of wax-retaining pulmonary implants in rats. Currently, the aim is to determine what other factors, deficiencies et al. will affect responses.

Secondly, we are investigating the critical factors responsible for fiber carcinogenesis in the lung and pleura which depend on structural configuration of the particles of fibers such as the gypsums, metal whiskers, talcs, asbestoses, and fibrous glasses and a variety of other durable fibers. An effort to develop a workable hypothesis on the primary effect of the fiber on cells is in progress.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1. Investigations into pulmonary carcinogenesis with tobacco products and other potential pulmonary carcinogens for man.

2. Investigations into the factors affecting development of mesotheliomas experimentally induced with asbestos, fibrous glass and related fibrous minerals.

I. Tobacco Carcinogenesis: Results of our initial tests with cigarette smoke condensate confirm that while the PAH fraction is by far the most carcinogenic component, other components, particularly the acidic fraction and aqueous methanol subfraction, augment carcinogenesis and are in themselves capable of pulmonary tumor induction. However, the fact that they augment pulmonary carcinogenesis would suggest that the removal of any of several components of either the acidic or neutral fractions of CSC might reduce carcinogenicity significantly. The results are also of significance in supporting the similar findings of skin painting experiments, thus reinforcing the idea that skin painting experiments are a valid means of determining pulmonary carcinogenesis.

This year Dr. Akagi completed and is in process of publishing a study on preneoplastic pulmonary squamous metaplasia. No qualitative differences are present in non-neoplastic and pre-neoplastic metaplasia although early breeching of the basement membranes and cellular anaplasia are early distinguishing features that herald the development of squamous carcinoma. We anticipate the initiation of a series of experiments on the influence of various local factors on tobacco induced pulmonary carcinoma such as the effects of TPA, nitrates, vitamin A analogs and polynuclear inhibitors. A large quantity of chemically characterized standard experimental blend III cigarette smoke condensate is available to us in small aliquots. It is planned that the Pharmacopathics contract will be primarily involved in these tests. Currently, this facility is involved in a test of extracted air pollutent fractions and seems fully utilized.

In the studies with intrapulmonary deposits of cigarette smoke condensates, a large series involving more than 4,000 rats has been initiated to determine the carcinogenic potential of 9 highly defined fractions of the PAH of cigarette smoke condensate isolated by the Agriculture Research Center, USDA, Athens, Georgia.

A large-scale contract, funded by DCCP to test the screening ability of the pulmonary pellet method to detect differences between cigarette smoke condensate from cigarettes with high and low nicotine content has been completed. Results indicate no effect from nicotine. In our own laboratory, we intend to test the method with several innovative cigarette smoke condensates and to explore the system with smoke condensates of other types that are common air pollutants. Briefly our plans call for testing nicotine and various fractions of cigarette smoke condensate and the condensate of cigarette smoke from cigarettes with altered or reduced tar and nicotine

as well as extracts from specific air pollutants and other potential pulmonary carcinogens that may be lipid soluble.

II. Fiber Carcinogenesis: In the area of fiber carcinogenesis, studies on glass are completed and published. We expect to complete and report on a large series of other types of fibers this year. Several projects have recently been initiated to understand the mechanism of fiber carcinogenesis. Facts currently known indicate that both large diametered fibers and minute short fibers are adequately handled by phagocytic activity, the latter by phagocytosis, the former by a process of fusion and plating the surface. However, fibers that have diameters <1.5 um and lengths greater than 8 μm are not touched by phagocytes. These long thin fibers subsequently initiate the deposition of extensive collagen and ultimately are the source of a high incidence of mesenchymal pleural neoplasms. The enigma of long thin fibers is in their avoidance of phagocytosis contrary to speculation in the past which presumed the scarring was the result of phagocyte deaths. We now have an in vivo system that should yield interesting information. Several activities are secondary to these studies. A review on possible effects of fibers on the gut was presented in March. Secondly, relevant to our mission concerning cancer diagnosis, I fully intend to explore the development of a system of electron probe analysis as a means of determining the nature of various pulmonary dusts and/or foreign bodies in other organs. Such efforts will require the close collaboration of Bill Banfield who has been developing this system for several years. Our input will consist of supplying tissues permeated with a variety of known fibers and other dusts. An atlas of wave length patterns from these studies of dusts can be developed which should serve in examining for unknown particles.

With the virtual completion of analysis of the response to fiber distribution of different sized fibers of three major types namely, aluminum oxide, dawsonite and glass, interest can now be turned to more refined considerations of mechanism. Several factors are apparent. Irrespective of the type of fiber, carcinogenicity directly relates to increasing lengths of fibers that are at the limit of optical variability or below. While it is easy to explain the lack of carcinogenicity in fibers sufficiently short to be completely entrapped by phagocytes, it is apparent that increasing length has an influence on carcinogenicity in fibers too long to relate to this phenomena. Several aspects of the situation seem involved in carcinogenicity. It is possible that carcinogenicity relates to surface contact with glass. Since finer fibers have far more surface area, one would expect a proportionately larger incidence of tumors with the finer fibers; however, it is unlikely that this could explain the complete lack of tumors in the controls implanted with large diametered glass. Another reasonable conjecture is that fiber ends are involved and that perhaps non-lethal penetrators of cell membranes is critical to carcinogenesis; however, if fiber ends were the critical factor one would expect proportionately higher numbers of tumors from moderately long fibers than from extremely long fibers. Since this does not seem to be the case, the problem remains.

Final data on these experiments should prove helpful in resolving these questions. However, the final resolution of this problem will probably depend on the results of experiments with fibers of precisely defined dimension which are in the process of being prepared from glass.

Regarding the screening of other materials, asbestos causes cancer not because it is asbestos, but because it contains durable fibers of exceptionally small diameter that are of exceptionally long length. The important corollary to this fact is that any durable fiber of comparable dimension may be as carcinogenic as asbestos. Our current efforts are being directed to searching and testing many types of fibers that are liable to contact with humans in one way or another. In particular, aerosols from dust-producing materials ranging from fertilizers to pollens as well as large diametered insulation materials are being examined. Furthermore, fibrous materials ingested orally are being studied. In general, testing consists of intrapleural application as previously described. However, we have recently begun a series of studies on the effect of intravenously administered fibers. This relates directly to the problem of orally ingested fibrous particles since absorption into the blood stream has been demonstrated in the rat.

Confirmation of the carcinogenicity of fibers for other species is also an item that will receive attention this year.

The effects of reduction in durability as a result of pyrolysis and the effect on surface properties by fiber coating will be investigated as time and space permit. Electron microscopy of candidate specimens of fibers and fine structure analysis of the early metaplastic alterations in pulmonary epithelium are in progress with the aim of understanding the preneoplastic changes that may be related to the induction of cancer.

A third aspect of fiber carcinogenesis relates to the effect of fibers on systems other than the lung. We have at hand a substantial group of rats that have received intravenously administered crocidolite. The tumor response in these animals is varied and complex. It will require considerable correlation with results of abundant long term controls which are also at hand and the good offers of Dr. Maxwell Layard, for analysis of variance.

A tabulation of all currently critical experiments completed and in progress is attached.

PLEURA EXPERIMENTS COMPLETED

		Pleural Sarcoma Probability %
1.	Tremolite (California)	100
2.	Dawsonite V (Alcoa)	100
3.	2d Fybex-2 (from JM)	100
4.	1st Fybex (from Zarth)	100
5.	40 mgs Silica Carbide Al_2O_3 GTC #1	100
6.	20 mgs Crocidolite + Gelatin	100
7.	Dawsonite I (JM)	95
8.	40 mgs UICC Crocidolite + Saline	87
9.	Dry Whole Fiberglass (JM 106)	77.8
10.	40 mgs Crocidolite + Gelatin	74.2
11.	Dawsonite VII (Alcoa)	68
12.	Dawsonite III (Synthetic)	66
13.	Dawsonite IV (Olduvai)	66
14.	200 mgs Cyclofil	60.2
15.	Microtomid AAA Glass, 40 mgs Shortfine	60
16.	Plastic Sheets, I (Pleura)	58.3
17.	4 mgs Aluminum Oxide GTC #3	44
18.	Al_2O_3-GE Type 4A Clusters	41
19.	40 mgs Mixed AlN + Al_2O_3	28
20.	40 mgs Aluminum GTC #2	22
21.	Refrasil Threads	21.7
22.	200 mgs Whole Yellow Glass	20.6
23.	Polyacrylic Nitrate	18.4
24.	Refrasil Dust	15.3
25.	Old Insulation Batting Glass Part. Pulv.	14.3
26.	Alcoa Dawsonite 6	13.0
27.	40 mgs Aluminum GTC #4	13.0
28.	200 mgs Mistron Vapor	12.7
29.	40 mgs Cotton Lint	12.6
30.	Dawsonite 2	12.0
31.	Attapulgite II	9.1
32.	Old Insulation Batting Glass, 40 mgs Whole	9.1
33.	Fiberglass Short-term (80 mgs)	8.4
34.	Synthetic Chrysotile JM	8.1
35.	Nickel Titanate	7.5
36.	40 mgs Cyclosorb JM	7.0
37.	40 mgs Fibrous Typsum JM	6.7
38.	40 mgs Fibrous Typsum JM	5.7
39.	40 mgs Steel Wool 000 Commercial	5.6
40.	Thermoflex with Chrome	5.2
41.	Diatomaceous Silica	5.2
42.	40 mgs Cyclofil JM	5
43.	40 mgs Aluminum Whiskers GTC #5	5
44.	40 mgs J & J Baby Talc JM	4.5

45.	40 mgs Mistron Frost JM	4.3
46.	Control, 80 mgs Yellow Glass Vehicle	2.4
47.	Thermoflex with Chrome	0
48.	Polyurethane Sponges	0
49.	40 mgs Partly Pulverized Commercial Vermiculate	0
50.	40 mgs Nytal 300 JM	0
51.	40 mgs Asbestine 5X JM	0
52.	40 mgs JM Mistron Vapor (Talc)	0
53.	200 mgs Asbestine 5X	0
54.	Fibrous Typsum JM	0
55.	Synthetic Chrysotile with 2% Cobalt JM	0

SUBCUTANEOUS EXPERIMENTS COMPLETED

1.	40 mgs Crocidolite + Glass	80.8
2.	Plastic Sheets (II)	67.2
3.	20 mgs Crocidolite + Tilantin	57.7
4.	40 mgs Crocidolite + Tilantin	43.6
5.	Yellow Vehicle Glass + Tilantin	0

PENDING EXPERIMENTS

		Experiment Starting Date
	Tremolite (Exp. 2) JM Code	9/3/75
	Tremolite (Series 2) JM	8/28/75
Book IV	FDA Chrysotile for I.V. Study 40 mgs	4/23/76
	Holloysite I	9/23/75
	Holloysite II	10/3/75
	Saffil II Grade Ziron	11/21/75
	Saffil I Alumina	12/4/75
	Carrogeerin	9/24/75
	Glass Wool + Silane	3/9/76
	Plastic Sheets-Polystyrene 2nd Exp., Group III	5/21/76
	Plastic Sheets-Perforated, Group IV	5/27/76
	Plastic Sheets-Scored, Group V	6/23/76
	Gelman Metrigard I	5/11/76
Book V	Control Yellow Vehicle Glass (108 Rats)	2/11/76
	Control Gelatin Only (102 Rats)	5/21/76
	Broken Yellow Vehicle Glass (52 Rats)	3/3/76

Publications:

1. Stanton, M.F., Layard, M.W., Tegeris, A., Miller, E., May, M., and Kent, B.: The carcinogenicity of fibrous glass: Pleural response in the rat in relation to dimension. J. Natl. Cancer Inst. 58: 475-487, 1977.

2. Stanton, M.F.: Can fibers in the gut cause cancer? J. Clin. Nutrition (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00516-12 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Comparative Studies on Neoplasms in Relation to Pathogenesis, Etiology and Diagnosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.F. Stanton	Medical Officer	LP NCI
OTHER:	G. Akagi	Guest Worker	LP NCI
	E. Miller	Biologist	LP NCI
	M. May	Biologist	LP NCI
	R.H. Adamson	Medical Officer	LCHPH NCI

COOPERATING UNITS (if any)

LCHPH, NCI
Pathological Technology Section, LP, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work centers on acquiring skills in the diagnosis and recognition of behavioral patterns in the tumors of laboratory animals employing techniques in light and electron microscopy and histochemistry. Major interest is currently focused on tumors of the Osborne-Mendel rat, since more than 4,000 aged females of this strain have been necropsied in this laboratory.

PHS-604D
(Rev. 10-76)

Project Description:

Objectives: A study and accessioning of cancer in laboratory animals. Collection and development of competence in special areas of cancer morphology. Studies of histopathological and fine structure alterations related to the pathogenesis of disease induced by oncogenic viruses and other carcinogens.

Major Findings: An ongoing program has been the development of special competence in the histopathological and fine structural aspects of tumors of the lung, bone and mammary glands of rodents. Collection and examination of induced and naturally occurring bone tumors has been in progress for more than 5 years and will continue.

The collection and development of competence in lung tumors is essential for the work previously described and additionally serves as an aid to other investigators in the area and to the NCI Registry of Experimental Cancers. A current project on which Ms. May serves is the study of the fine structure of the early, squamous metaplasia preceding carcinoma at the site of implanted carcinogen pellets and the comparison of this change with the metaplasia associated with non-specific pulmonary inflammation which does not progress to cancer.

Interest in mammary neoplasia in rats is an offshoot of current long-term pulmonary experiments. Since over 50% of female strain OM rats develop mammary tumors in the second year of life it is necessary to surgically remove these in our experimental groups. As a result we have correlated data on incidence, growth rate and morphology in several hundred excised mammary neoplasms.

Proposed Course: Tumors of all types will be transplanted into inbred rats in an effort to learn more about specific tumors. We have through the past 5-6 years acquired an extensive catalog of neoplasms of all types that develop in aged female Osborne-Mendel rats. This data will be collated in an effort to establish normal incidence rates for our colony. In addition, time will be devoted to training in electron microscopy, studies of neoplasia in lower animals and preparation of a monograph on lung tumors in rats.

Publications:

1. Stanton, M.F.: Tumors of bone. WHO Monograph Pathology of Tumours in Experimental Animals, vol. II, Tumours of the Mouse. (in press).

2. Stanton, M.F.: Tumors of the lung. WHO Monograph Pathology of Tumours in Experimental Animals, vol. I, pt. II, Tumours of the Rat. (in press).

Publications (Cont'd)

3. Dawe, C.J., Sonstegard, R., Stanton, M.F., Woronecki, D.E., and Reppert, R.T.: Intrahepatic biliary duct neoplasms in Catastomus commersoni. In Homburger, F. (Ed.): Progress in Experimental Tumor Research. Basel, S. Karger, 1976, vol. 20, pp. 195-204.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00521-07 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Influence of Epithelial-Mesenchymal Interactions on Neoplastic Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.J. Dawe	Medical Officer	LP NCI
OTHER:	J. Peng	Medical Officer	MO NCI
	Y. Kurokawa	Visiting Fellow	LP NCI
	W.D. Morgan	Biologist	LP NCI
	J.E. Williams	Biologist	LP NCI
	C.M. Poore	Biologist	FCRC NCI
	T. Sakakura	Former Visiting Fellow	LP NCI (now at Aichi Inst. for Cancer Research, Nagoya, Japan)

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is a continuing investigation of the effects of interactions between epithelium and stroma during the transformation of epithelium from normal to neoplastic state. The systems currently being studied are: 1) Tumors of the submandibular salivary gland of mice, induced by polyoma virus; 2) Tumors of the forestomach and glandular stomach of mice, induced by chemical carcinogens; 3) Mammary tumors of mice, spontaneous or induced by mammary tumor virus (MTV). The general methods used involve neoplastic transformation of epithelium in the presence or absence of specific types of mesenchyme (stroma). The results are evaluated on the basis of morphological and biological properties of the resulting neoplasms.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Most neoplasms, like normal tissues, show the ability of their stem cells to undergo some degree of differentiation and to manifest some amount of functional activity. Developmental biologists have shown that an important determinant in normal morphogenesis and differentiation of glandular organs is the interaction between the epithelial and mesenchymal components. Therefore it should follow that the nature of epithelial-mesenchymal interaction in progress when neoplastic transformation of epithelium takes place will in some degree affect the kind and the extent of differentiation of the resulting tumor. The object of this project is to test this hypothesis and to determine whether interference with epithelio-mesenchymal interactions can modify the effectiveness of carcinogenic agents.

Methods Employed: Work with the 3 systems listed above is separately described below, for each.

A. Polyoma virus/mouse salivary gland system.

The general approach is to separate embryonic rudiments of salivary glands into epithelial and mesenchymal components by means of enzymatic (pancreatic extract) dissection of the rudiments. The components are then infected with polyoma virus, either separately or in one of several configurations in relation to mesenchyme, as indicated below. The use of Millipore filters as artificial permeable barriers between epithelium and mesenchyme, or as simple physical support for either type of tissue alone is the technique currently in use. Previously published studies using recombinations in which epithelium and mesenchyme were in direct contact without a filter barrier form the basis for comparison with trans-filter and simple epi-filter experiments.

1. Salivary epithelium opposite salivary mesenchyme - with and without polyoma virus.

2. Salivary epithelium opposite salivary epithelium - with and without virus.

3. Salivary mesenchyme opposite salivary mesenchyme - with and without virus.

4. Salivary epithelium alone, on one side of membrane - with and without virus.

5. Salivary mesenchyme alone, on one side of membrane - with and without virus.

Cultures of these types were maintained for periods up to a year, in modified culture chambers fitted with polyethylene tubes for exchange of nutrients and gases. At intervals, the Millipore membranes were removed and results evaluated by 1) light and electron microscopy of the cells on the membranes; 2) observations of the behavior of the recovered cells in Leighton tube cultures; 3) observation of the tumorigenic capacities of the cells after subcutaneous transfer to newborn mice; 4) tissue culture infectivity tests for continued presence of PV in the transformed cells; 5) fluorescent antibody tests for polyoma T antigens in transformed cells (these tests were done by Mrs. Yee using FA prepared by Dr. K. Takemoto).

Major Findings: Data from experiments in which epithelium and mesenchyme were placed opposite each other on filters have been published, and the outlines of results with PV-infected epithelium alone on filters are becoming visible, as indicated in the attached table.

Control cultures not infected with PV failed to undergo transformation.

With FA vs. polyoma T antigen, the transformed cells in all cell lines except 1 were positive, and all lines are carriers of actively replicating virus. Repetition of the T-antigen test on the exceptional line should be done, as this test is sometimes negative even with known polyoma transformants.

The comparisons above show strong differences between tumors induced in salivary epithelium transformed either in complete isolation from mesenchyme, or separated from mesenchyme by a filter. The neoplasms obtained in culture transformations were more undifferentiated and more aggressive biologically.

Significance: This experiment demonstrates that epithelial-mesenchymal interaction is not necessary for epithelial transformation to take place under the influence of PV. However, it shows that the microenvironment of PV-infected cells at the time of transformation and/or thereafter (the time factor here may be of importance) is strongly influential in determining the morphological and biological properties of the transformed cells.

Since invasiveness and metastasizing ability are clinically important features of neoplasms, it seems worthwhile to investigate the mechanisms through which epithelial-mesenchymal interaction modifies the outcome of neoplastic transformation in a seemingly favorable way from the standpoint of the host.

Proposed Course: An additional set of controls has been added to the experiment, consisting of culture lines of PV-induced salivary tumors *in vivo*. These lines are being isolated by placing tumor cells on Millipore filters, growing them for periods of 30 days to 1 year on filter, and then transferring to standard glass tubes. In this way the entire transformation experiment is recapitulated except that the culture conditions are imposed

only after transformation, not during it. In this way it will be possible to determine whether the differences observed are the results of progression in vitro, or basic differences in the primary transforming events.

B. Dr. Yuji Kurokawa's project: A study of epithelial-mesenchymal interaction during chemical carcinogenesis in glandular and squamous portions of the stomach.

Methods Employed: These experiments will be concerned with observations on tissues undergoing neoplastic transformation in vivo, although some of the manipulative procedures and exposure of tissues to carcinogens must be done in vitro.

The general experimental plan is to remove stomachs from embryo C3H/Bi mice at 15 days of gestation, and to transpose the mesenchymes of the glandular and squamous portions of the stomachs prior to exposing the recombinations to chemical carcinogens. The carcinogen-treated recombinations are then transplanted to syngeneic mice, and when tumors develop in these transplants, they are compared histologically and biologically with tumors induced in controls. Controls consist of (a) intact glandular and squamous portions of the 15-day embryonic stomach, treated with the same carcinogen and transplanted to syngeneic mice; (b) recombinations of glandular stomach epithelium with glandular stomach mesenchyme, and of squamous stomach epithelium with squamous stomach mesenchyme, treated with the same carcinogen and transplanted to syngeneic mice. In addition, some experiments may be done in which the mesenchyme alone will be exposed to carcinogen. After a time sufficient to allow metabolism or degradation of the carcinogen, mesenchyme will be recombined with epithelium and observed in transplant for appearance of tumors.

By these procedures it should be possible to determine whether "phenotypic hybridization" of epithelial and mesenchymal components of an organ can alter the ability of the epithelium to respond to a given carcinogen, and the type of response the epithelium makes in such a changed stromal environment. For example, it might be postulated that a combination of glandular mesenchyme with squamous (forestomach) epithelium would give rise to adenocarcinoma rather than to squamous carcinoma. Such a postulate can be tested by these experiments, although it should be noted that we would not expect this particular postulate to prove correct.

Dr. Kurokawa has worked out the techniques for excision of stomachs from 15-day embryo mice, for recognizing and separating the glandular and squamous portions, and for separating the mesenchyme and epithelium of each portion and recombining as desired in short-term tissue cultures. He has also learned the methods for transplanting the recombinations into either newborn or adult mice, and for finding these transplants several weeks later. In other words, he has acquired all the techniques needed to execute the experimental plan.

Major Findings: Dr. Kurokawa is currently just concluding his experiments by necropsy study of his mice. Until the necropsies are completed at the end of another 3 months, the results will not be available.

Proposed Course: This will be decided upon after the results are in. Meanwhile, Dr. Kurokawa has been preparing a literature review on the subject of epithelial-mesenchymal interactions in relation to tumor development. Dr. Kurokawa will remain as a Visiting Scientist until October 9, 1977, without departmental salary during that period, but with full institutional support and provision of facilities and resources.

C. Dr. Teruo Sakakura's project: A study of epithelial-mesenchymal interactions in relation to normal development and tumor development in mouse mammary gland.

This project was started during Dr. Sakakura's 1-year sojourn as a Visiting Scientist in 1974-75. After her return to the Aichi Institute for Cancer Research in Nagoya, Japan, she completed a portion of the work started here, and will continue extensions of that work, on her own and with continued suggestions from the PI.

The completed work was published during the past year in Science (see reference below), and is briefly summarized as follows: Recombinations of mammary epithelium with salivary mesenchyme were found to develop in the fashion of salivary gland with respect to geometrical features. However, with respect to biochemical features, these recombinations behaved like mammary gland in that -lactalbumin, a component of milk, was secreted under the stimulus of the hormones of pregnancy and the puerparium.

Dr. Sakakura plans to make use of similar "mismatched" epithelio-mesenchymal recombinations to determine whether they predispose to, or possibly protect against, carcinogenic stimuli.

Publications:

1. Dawe, C.J., Morgan, W.D., Williams, J.E., and Summerour, J.P.: Inductive epithelio-mesenchymal interaction: Is it involved in the development of epithelial neoplasms? In Müller-Bérat, N. (Ed.): Progress in Differentiation Research. New York, Amer. Elsevier Pub. Co., Inc., 1976, pp. 305-318.

2. Sakakura, T., Nishizuka, Y., and Dawe, C.J.: Mesenchyme-dependent morphogenesis and epithelium-specific differentiation in mammary gland. Science 194: 1439-1441, 1976.

Epithelial Transformation, Ep/Mes (3 lines)	Epithelial Transformation, Ep/Ep or Ep/o (4 lines)	Epithelial Transformation, in vivo or in organ culture
1. Pleomorphic, highly aneuploid, poorly differentiated	Poorly differentiated (karyotyping incomplete)	Mixed epithelial and mesenchymal appearance, but only slightly aneuploid
2. Invasive, often metastasizing	Invasive	Non-invasive, non-metastasizing, well-encapsulated
3. Sometimes with areas of either squamous or adenocarcinoma	No squamous or adeno component	No squamous component. Adeno component often
4. Rarely, bone-forming activity as well as epithelial structure	No bone formation	No bone formation

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00538-12 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Phylogenetic Aspects of Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.J. Dawe	Medical Officer	LP NCI
OTHER:	W.G. Banfield	Medical Officer	LP NCI
	J. Peng	Medical Officer	MO NCI
	R. Sonstegard	Medical Officer	Univ. of Guelph, Ontario
	C.M. Poore	Biologist	FCRC NCI
	S.R. Wellings and associates	Medical Officer	Univ. of Calif., Davis

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	5	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a continuing study in comparative oncology, designed to identify and characterize neoplasms in lower animals including cold-blooded vertebrates and the invertebrates. By studying neoplasms in animals phylogenetically more remotely removed from man, it is hoped to gain a more complete understanding of the basic biology of neoplasia at all phylogenetic levels. It is expected that such studies will reveal properties of neoplasia not previously suspected to exist because of the excessively narrow scope of studies limited to the higher animals and man. Important differences in the immunologic, biochemical, and regenerative characteristics of the lower animals should serve to emphasize the relevance of these characteristics to the development of and the susceptibility to neoplasia. In addition, by study of neoplasms in lower animals under feral conditions, these neoplasms may be found to serve as indicators of environmental tumorigenic agents. Neoplasms are collected, either in the field or through submittal from outside sources, and are studied morphologically and biologically. Light and electron microscopy, tissue culture, transplantation, and tumor induction methods are applied.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: During the past year 1976-77, investigations were centered on 2 projects:

A. A pathological anatomical study of tumors of the pseudobranchial (parabranchial) body in the Pacific gray cod.

B. An ultrastructural and light microscopic study of the neoplastic cells in malignant lymphoma of northern pike.

These 2 projects are summarized separately below.

Project A. Methods Employed: Tumors of the parabranchial bodies, prevalent in as many as 40 percent of intermediate- and large-sized cod along the North American Pacific coast, were sent to us by Mr. J. Bagshaw, Fisheries and Marine, Environment Canada, Pacific Biological Station, Nanaimo, British Columbia, Canada. These tumors and normal pseudobranchial bodies, as controls, were examined by light microscopy of sections subjected to histochemical reactions and routine staining methods, and by transmission electron microscopy. The objective was to determine the normal cell type of origin of these tumors and to characterize the tumors morphologically as completely as possible.

Major Findings: Pseudobranch tumors were unexpectedly found to be composed chiefly of an unusual cell type previously observed in large numbers in cutaneous papillomas in flounders along the Pacific coast. This cell type had previously been noted to have some features of unicellular microorganisms, and had been designated "X cell" by Brooks and Wellings and colleagues in 1969.

X cells in the pseudobranch tumors in cod were characterized by large size, spherical shape, a nucleus containing a single large, dense nucleolus; fine dispersal of nuclear chromatin, intracytoplasmic, membrane-bound dense granules that gave an argyrophilic reaction with the Sevier-Munger silver method; small, poorly developed mitochondria; and a conspicuously heavy coat, or glycocalyx, on the plasma membrane. Nuclei of X cells gave negative reactions to the standard Feulgen technique for DNA, reflecting the dispersed state of nuclear chromatin. Large numbers of X cells were enveloped by thin, greatly extended cytoplasmic processes of normal host epithelial cells.

In view of these findings, it is considered highly probable, but not conclusive, that X cells, both in flounder papillomas and in cod pseudobranch tumors, are parasitic cells rather than neoplastic cells. The chief feature of X cells possibly arguing against the parasitic concept is the membrane-bound dense granules in X cell cytoplasm. These granules are similar to secretory granules in polypeptide hormone-secreting cells, and might indicate that the flounder papillomas and cod pseudobranch tumors originate from chemoreceptor cells.

Significance: It is of some importance to determine with finality whether X cells are parasites or tumor cells, because until now the high prevalences of flounder papillomas and cod pseudobranch tumors have been widely supposed to be related to some environmental carcinogen(s). If these "tumors" are in reality parasitic diseases, many researchers have been working on the basis of a false premise. At the same time, if X cells are parasites and can mimic tumors effectively, they may deserve study to determine whether X cells produce something comparable to tumor angiogenesis factor that could account for the vascular proliferation associated with flounder papillomas.

Proposed Course: Discussions with Dr. Peter Howley and Dr. Chester Herman have led to a plan that should succeed in deciding whether X cells are parasites or tumor cells. We have enlisted the aid of Dr. Wellings to provide frozen and fixed specimen materials, and have forwarded him a collecting and shipping list that should assure the arrival of specimens in good condition. The methods to be applied are flow fluorimetric analysis (Dr. Herman) and melting point curve determinations of X cell DNA as compared with melting point curves of normal host cell DNA. In addition, Dr. Dawe plans to conduct cell culture studies of X cells that may be of further use in identifying the nature of X cells.

This project is a collaborative one involving the group of investigators under Dr. Wellings at the University of California and several members of the Laboratory of Pathology at NCI. In the event that Drs. Howley and/or Herman complete studies using the methods listed above, they would be Principal Investigators of their respective projects. Dr. Dawe is merely acting as a coordinator to extend the study of X cells beyond the original study in which he participated.

Project B. Objectives: This is a cytological study of the neoplastic cells in the epizootic malignant lymphoma of northern pike. It is actually a collaborative study deriving from initial investigations on the pike lymphoma by Dr. R. Sonstegard, University of Guelph, Guelph, Ontario. The main objective was to characterize the lymphoma cells by light and electron microscopy, in extension of a similar study on lymphomas in northern pike in Ireland, completed several years ago in collaboration with Dr. Maire Mulcahy and Dr. Gösta Winqvist.

Methods Employed: Standard methods of light and transmission electron microscopy were used, including examination of lymphoma cells in tissue culture by Wright's-stained smears and by TEM of centrifuged pellets of culture cells. In addition, Dr. Jacqueline Peng did karyotype analyses on metaphase figures of lymphoma cells, using standard cytogenetic techniques.

Major Findings: At the light microscopic level the lymphoma cells were classified as undifferentiated in type. Two structures not previously noted in the Irish pike lymphomas were observed: 1) "Nucleoid bodies", which were spheroidal or elliptical masses of chromatin-like material in the cytoplasm. These ranged in size up to that of a small lymphocyte

nucleus. They were relatively rare and could not be found with sufficient certainty by electron microscopy to characterize them. 2) "Cylindroid lamella-particle complexes", comparable to "ribosome-lamella complexes" found in hairy-cell leukemia, monoblastic leukemia, chronic lymphocytic leukemia, lymphosarcoma cell leukemia, and Waldenström's macroglobulinemia in man. These structures have now been fully described in publications in the references listed. They have been postulated by others to represent virus arrays or components, but our studies have given no evidence of virus particles in the lamella-particle complexes.

A cytogenetic study of pike lymphoma cells was completed by Dr. Peng in collaboration with Drs. Sonstegard and Dawe (see ref. below). It revealed that the pike lymphomas in North America regularly have a submetacentric marker chromosome, 1 minute chromosome, and 3 to 5 pairs of smaller than normal acrocentric chromosomes. This pattern differs slightly from that seen in pike lymphoma in Sweden, where 2 or 3 submetacentric marker chromosomes are usually present. The North American pike lymphoma is an outstanding example of a naturally occurring neoplasm with a karyotypic "signature".

Significance: Although cylindroid lamella-particle complexes were once thought to be pathognomonic of hairy cell leukemia in man, it is now known that they may be found in a variety of neoplasms and even in some apparently normal cells in man. Their discovery in pike lymphoma provides an animal source for further study of these enigmatic structures. It currently seems improbable that they are directly related to neoplastic processes or oncogenic viruses.

The origin and biological significance of nucleoid bodies in pike lymphoma cells are completely unknown. They may represent chromosomal "discards" related to the constant chromosomal anomalies found in the pike lymphoma.

The discovery by Dr. Peng of a karyotypic "signature" in the pike lymphoma appears to parallel the finding of the Ph^1 chromosome associated with chronic myelogenous leukemia in man. This may signify some inherent genetic susceptibility to develop lymphoma. However, other evidence (temperature-sensitive reverse transcriptase) suggesting viral etiology of the pike lymphoma may be interpreted to mean that a viral agent induces a constant pattern of chromosomal changes in the cells it transforms.

Proposed Course: For the coming year, no further work with the pike lymphoma is planned. However, Dr. Banfield is continuing to make a search for lamella-particle complexes in various normal and neoplastic tissues.

Project C: Intrahepatic biliary duct neoplasms in white suckers (Catastomus commersonii).

This project was concluded with the publication cited below. No further work is contemplated in this area in the immediate future.

Publications:

Project A

1. Alpers, C.E., McCain, B.B., Myers, M., Wellings, S.R., Poore, M., Bagshaw, J., and Dawe, C.J.: Pathological anatomy of pseudobranch tumors in Pacific cod, Gadus macrocephalus. J. Natl. Cancer Inst. (in press)

Project B

1. Banfield, W.G., Dawe, C.J., Lee, C.W., and Sonstegard, R.: Cylindroid lamella-particle complexes in lymphoma cells of northern pike (Esox lucius). J. Natl. Cancer Inst. 57: 415-420, 1976.

2. Dawe, C.J., Banfield, W.G., Sonstegard, R., Lee, C.W., and Michelitch, H.J.: Cylindroid lamella-particle complexes and nucleoid intracytoplasmic bodies in lymphoma cells of northern pike (Esox lucius). In Homburger, F. (Ed.): Progress in Experimental Tumor Research. Basel, S. Karger, 1976, vol. 20, pp. 166-180.

3. Whang-Peng, J., Sonstegard, R.A., and Dawe, C.: Chromosomal characteristics of malignant lymphoma in northern pike (Esox lucius) from the United States. Cancer Res. 36: 3554-3560, 1976.

Project C

1. Dawe, C.J., Sonstegard, R., Stanton, M.F., Woronecki, D.E., and Reppert, R.T.: Intrahepatic biliary duct neoplasms in Catastomus commersoni. In Homburger, F. (Ed.): Progress in Experimental Tumor Research. Basel, S. Karger, 1976, vol. 20, pp. 195-204.

Related reviews and editorial work:

1. Dawe, C.J.: Spontaneous neoplasms in fishes. In Chapter 1 "Tumors". In Pathology of Laboratory Animals, Vol. 2, Benirschke, K., Garner, F.M., and Jones, T.C. (Eds.) Springer-Verlag, New York.

2. Kraybill, H. et al (eds.): Annals N.Y. Acad. Sci.: Aquatic pollutants and biological effects with emphasis on neoplasia. (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00540-02 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Contractile Tissues in Organ Cultures of Mouse Submandibular Glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.D. Morgan	Biologist	LP NCI
OTHER:	C.J. Dawe	Medical Officer	LP NCI
	C.W. Lee	Biologist	LP NCI
	J.E. Williams	Biologist	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	1	3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Using time-lapse photographic techniques we have identified three types of contractions in mouse submandibular gland rudiments in organotypic culture. The first and second types caused central and peripheral portions of the gland to contract synchronously. The third type caused the gland to contract asynchronously. Each type of contraction was given a functional name. The first type, which had a latency period that ranged from 30 minutes to 6 hours and appeared to purge the ducts and acini of their contents, is called voiding contraction. The second type, which had a latency period that ranged from 4 to 60 minutes and appeared to force the contents of distal tubules toward main ducts, is called priming contraction. The third type, which had a latency period that ranged from 5 to 14 minutes and appeared to whip acini randomly in a twitching manner, is called churning contraction. A preliminary film of these contractions will be presented at the 28th Annual Meeting of the Tissue Culture Association at New Orleans in June, 1977. Subsequently, a documented film of contractions in mouse submandibular gland, as revealed by time-lapse photography will be completed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of this project will be 1) to produce a documentary film that clearly shows contractions in organ cultures of mouse embryo submandibular glands; 2) to determine whether the contractile element(s) observed in mouse submandibular glands appear to be myoepithelium or smooth muscle; and 3) to determine whether parasympathetic nerve elements in these glands affect contractions observed there.

Methods Employed: Organ cultures of submandibular glands of 14 day embryo mice will be cultured on glass in modified Rose tissue culture chambers and in tube cultures. Chamber cultures will be photographed, using techniques of time-lapse photography. Tube cultures will be studied by histochemical and electron microscopic techniques. Glands with and without their submandibular ganglia will be studied. Time-lapse photography will be used to make a permanent record of contractions; while histochemistry and electron microscopy will be used to identify components of myoepithelium, smooth muscle, and parasympathetic nerve.

Major Findings: Contractions in organ cultures of 14 day embryo mouse submandibular glands began approximately 6 days after start of culture and continued for more than 30 days. Time-lapse photography show the contractions to be mixed: some appeared synchronous and occurred frequently, and others appeared asynchronous and occurred infrequently.

Significance: Contractile cells such as myoepithelium play a significant roll in some tumors of salivary glands (pleomorphic adenomas or so-called mixed tumors, for example). A documentary film on the action of these cells in normal glands may increase our understanding of the role played by contractile cells in neoplasms of salivary glands.

Proposed Course: The results obtained from this project will be described and illustrated in a written report and further illustrated in a documentary film.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00560-11 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Electron Probe and Scanning Electron Microscope

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.G. Banfield	Medical Officer	LP NCI
OTHER:	S.B. Doty	Medical Officer	LBS NIDR
	E.G. Trams	Medical Officer	LNC NINCDS
	C.J. Lauter	Medical Officer	LNC NINCDS
	C.W. Lee	Biologist	LP NCI

COOPERATING UNITS (if any)

LNC, NINCDS; LBS, NIDR

LAB/BRANCH

Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this work is to develop the instrumentation and techniques so that the x-ray and other analytical capabilities of the scanning electron microscope can be applied effectively to biological problems. The subjects of present interest are elastin and stratified squamous epithelium.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To develop the instrumentation and techniques necessary to apply the electron probe and scanning electron microscope to the analysis of biological specimens.

Major Findings: 1. Magnetic tape drives for storing and entering programs and spectra have been incorporated into the energy dispersive x-ray analytical system of the ETEC scanning electron microscope.

2. The National Bureau of Standards FRAM program for quantitative analysis using an energy dispersive detector is now operating on the PDP10 computer.

Significance: The tape drive allows for more effective use of the EDAX system.

The FRAM program is the first reliable documented program written for quantitative analysis using the energy dispersive system and which can be put on a relatively small dedicated computer - 32K of memory.

Proposed Course:

1. Upgrade the Nova 1210 computer in the EDAX system to 32K of memory by the addition of 24K of memory and incorporate a Data General Diskette subsystem.

2. Adapt a new version of the NBS program FRAM to the PDP10 and as soon as the EDAX system is upgraded put it on the Nova 1210 computer.

3. Construct a transmitted light microscope stage for use with the x-ray analysis equipment on the scanning electron microscope.

Publications:

1. Trams, E.G., Lauter, C.J., and Banfield, W.G.: On the activation of plasma membrane ecto-enzymes by treatment with neuraminidase. J. Neurochem. 27: 1035-1042, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00561-07 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Collaborative Research and Other Activities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M.F. Stanton Medical Officer LP NCI

COOPERATING UNITS (if any)

None

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Laboratory of Pathology

SECTION

Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fifteen percent of the normal work week is spent in the following activities: 1. Member, Smoking and Health Review Board-OD; 2. Member, Asbestos Protocol Subcommittee of the HEW Committee on Coordinated Toxicological Research and Related Programs; 3. Project Officer, NCI, DCBD-NO1-CB-33887, Small Animal Holding Facility; 4. Project Officer, NCI, DCBD-NO1-CB-43912, Hematoxylin Substitute Development; 5. Co-Project Officer, NCI, DCBD-NO1-CB-33874, Registry, Tumors in Lower Animals; 6. Co-Project Officer, NCI, DCCP-NO1-CP-3374, Standardized Lung Pellet Technique; 7. Member, Editorial Board Monograph Series, IARC-WHO, Lyon, France; 8. Member, ILARS Subcommittee on Tumors in Laboratory Animals, NRC; 9. Dept. of Commerce, Nat. Bureau of Standards Working Group on Establishment of Definitions and Criteria for Mineral Fibers; 10. Associate Editor, Journal of Environmental Pathology & Toxicology; 11. Associate Editor, Journal of Comparative Pathology.

PHS-6040
(Rev. 10-76)

Summary of Work (Cont'd)

These activities primarily involve investigations into the carcinogenicity of asbestos, other durable fibers, and tobacco products. Additionally they involve the collection of data on neoplasms in laboratory and histochemical investigations as an aid to diagnosis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00593-22 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

An Investigation of the Collagenous Connective Tissues and Elastin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.G. Banfield	Medical Officer	LP NCI
OTHER:	L. Sokoloff	Medical Officer	State Univ. of N.Y.
	C.W. Lee	Biologist	LP NCI
	A. Hough	Medical Officer	Vanderbilt Univ.

COOPERATING UNITS (if any)

LEP, NIAMDD; Dept. of Path., Vanderbilt Univ.; and Dept. of Path., Health Sciences Center, State Univ. of New York, Stony Brook, N.Y.

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Laboratory of Pathology

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Comparative Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

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4	3	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this continuing study of connective tissues the joint cartilage in hemophilic arthropathy has been examined using the scanning electron microscope equipped with an energy dispersive x-ray analyzer. Now the effort will be directed toward the chemical and morphologic changes in elastin during the development of "senile elastosis".

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To elucidate the normal and abnormal structure of elastin and collagen and their associated cellular and intercellular components.

Major Findings: 1. In collaboration with Drs. Hough and Sokoloff an EDAX (energy dispersive analysis by x-rays) study of joint cartilage in hemophilic arthropathy using the scanning electron microscope as an electron probe has been published. Abnormal inclusions containing iron associated with phosphorus are present within the chondrocytes in advanced hemophilic arthropathy.

2. Review paper for a Hayat Monograph was accepted.

Significance: Demonstration of the potential of the scanning electron microscope, when used as an electron probe, to perform non-destructive elemental analysis on microscopic structures.

Proposed Course: Apply the scanning electron microscope with the energy dispersive x-ray analyzer to the analysis of connective tissues, particularly elastin and elastotic degeneration (senile elastosis). Tissue has been collected on which to perform the latter study.

Publications:

1. Hough, A.J., Banfield, W.G., and Sokoloff, L.: Ultrastructural and microanalytical studies of cartilage in hemophilic arthropathy. Arch. Pathol. 100: 91-96, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00533-04 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Oncogenic DNA Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.C. Costa	Medical Officer	LP NCI
OTHER:	A.S. Rabson	Medical Officer	DCBD NCI
	T.J. Triche	Medical Officer	LP NCI
	Y. Nakamura	Visiting Fellow	LP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology & Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NJH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general scope of this project is to study biological properties of human herpesviruses and human papovaviruses tentatively associated with tumors in human patients. At present the following topics are under investigation. 1) Studies on the Fc receptor induced by herpes simplex virus; 2) Characterization of the interaction of a human papovavirus with human cells in vitro.

PHS-6040
(Rev. 10-76)

Project Description:

1) To characterize the properties of the Fc receptor induced by herpes simplex virus (HSV). Work in our laboratory reveals that the synthesis of infectious virus is inhibited by the presence of rabbit IgG and we have found that viral DNA synthesis is also markedly decreased in the presence of IgG. We have isolated mutant strains of HSV that are IgG resistant.

2) A human tumor cell line derived from a glioblastoma multiforme was established. After injection with BK(MM)V, a human papovavirus, the cell cultures behaved as supertransformants but after a period of months started synthesizing late viral antigens. This indicated some leakiness of the viral transformation. We plan to analyze the status of the viral genome of BK(MM)V in the cell line.

Proposed Course:

1) Experiments are in progress to: a) characterize the effect of rabbit IgG on HSV replication by finishing the experiments related to the DNA inhibition and determining changes in the patterns of viral peptides synthesized in the infected cells; b) to determine if internalization of the bound IgG is necessary for the effect; c) to determine if the inhibitory effect upon HSV is viral specific or affects other viruses (interference); d) to determine if the Fc receptor sites are mixed with other viral antigens at the cell surface or exist in "segregated particles"; e) to characterize the IgG resistant strains; f) to purify the Fc binding molecule.

2) The presence of viral antigens in the cells will be determined by immunofluorescence. The presence of viral genome and its relationship to the host DNA will be determined. Different clones of cells will be analyzed.

Publications:

1. Costa, J., Yee, C., Tralka, T.S., and Rabson, A.S.: Hamster ependymomas produced by intracerebral inoculation of a human papovavirus. J. Natl. Cancer Inst. 56: 104-105, 1976.

2. Costa, J.C., Howley, P.M., Legallais, F.Y., Yee, C.L., Young, N.A., and Rabson, A.S.: Oncogenicity of a nude mouse cell line transformed by a human papovavirus. J. Natl. Cancer Inst. 58: 1147-1150, 1977.

3. Costa, J., Rabson, A.S., Yee, C., and Tralka, T.S.: Viral growth inhibition by immunoglobulin binding to herpesvirus-induced Fc receptors. Nature (in press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00534-03 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Structure and Assembly of Virulent and Vaccine Polioviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: N.A. Young Medical Officer LP NCI

COOPERATING UNITS (if any)

James Nakano, Enterovirus Unit, Center for Disease Control
Atlanta, Georgia

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/3	2/3	2/3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The structural lability of the largest virion polypeptide (VP1) of type 1 poliovaccine has been used to identify mutations occurring in virus strains isolated from human feces. Following vaccine feeding, a mutant of the vaccine is progressively selected after several replication cycles in the gut. This mutant, which has altered antigenic properties and has regained the capacity to synthesize a stable VP1, may be responsible for rare instances of vaccine-associated paralytic disease.

PHS-6040
(Rev. 10-76)

Project Description:

Virion polypeptides of the type 1 Sabin vaccine poliovirus (LSc2ab) were analyzed by SDS gel electrophoresis. A lesion occurring during early assembly caused the largest polypeptide, VP1, to be degraded in the presence of SDS. In contrast VP1 of the parent neurovirulent strain (Mahoney) and an unrelated type 1 neurovirulent strain (Brunhilde) was stable. Since strains of poliovirus isolated from feces of Sabin vaccine recipients are often intermediate between vaccine and wild with respect to neurovirulence, rct 40, and antigenic markers, we assessed the potential application of the polypeptide lesion to the identification of human isolates. Eight type 1 isolates of known pedigree, received as coded unknowns, were correctly identified as vaccine or wild by analysis of polypeptide profiles.

Similar analysis performed on 5 strains isolated sequentially from feces of a child fed LSc2ab vaccine showed increasing amounts of VP1 in each successive isolate, although all remained distinguishable from naturally-occurring virulent strains. Cloning experiments revealed that each isolate represented a mixed population of viruses consisting of 1) vaccine virus with unstable VP1; 2) mutated vaccine virus with a stable VP1 migrating faster than wild type VP1. A virus recovered from a case of vaccine-associated paralytic disease in an immunodeficient host was shown to have the polypeptide profile of mutated vaccine virus, strongly suggesting that the mutant may possess modestly increased neurovirulence compared with reference vaccine virus. These results indicate that mutation(s) resulting in instability of VP1 of attenuated virus are "leaky" after back-passage in man.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00535-02 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biologic and Virologic Characterization of Breast Cancer Cell Lines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N.A. Young	Medical Officer	LP NCI
OTHER:	J.A. Strauchen	Clinical Associate	LP NCI

COOPERATING UNITS (if any)

Marc Lippman, M.D., Medicine Branch, NCI

LAB/BRANCH

Laboratory of Pathology

SECTION

Viral Oncology and Molecular Pathology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2/3	1/3	1/3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

One additional line of breast cancer cells has been established in continuous culture, making a total of 4. On serial passage these cells continue to exhibit stable karyotypic abnormalities and responsiveness to estrogen and other steroid hormones, but synthesis of alpha lactalbumin can no longer be demonstrated. Oncornavirus expression has not been detected.

In parallel experiments with a murine cell line stimulated by dexamethasone, mouse mammary tumor virus expression was specifically inhibited by interferon. The results suggested a block at the level of viral assembly or release from the cell membrane.

PHS-6040
(Rev. 10-76)

Project Description:

Studies of the possible role of oncornaviruses in the etiology of human breast cancer have been hindered by the lack of well-characterized pure populations of breast cancer cells. We have processed cells from malignant effusions and have succeeded in developing 4 continuous lines. Investigations of these cells have developed in 2 major directions: 1) biologic characterization of the cells and 2) a search for evidence of oncornavirus expression. Major findings to date on one of these cell lines which has been the most thoroughly studied include stable karyotypic abnormalities; ultrastructural features compatible with secretory epithelial cells; synthesis of differentiated proteins such as alpha lactalbumin and casein; presence of receptors for estrogen and other steroid hormones; growth responsiveness to estrogen and insulin. No complete (infectious) oncornaviruses have been detected in these cells as assayed by the presence of 70S RNA in particles banding at p 1.16 - 1.18; reverse transcriptase activity; electron microscopy; co-cultivation with a variety of human and animal indicator cells.

In parallel experiments on murine cells stimulated with dexamethasone, which specifically induces synthesis of mouse mammary tumor virus (MMTV), we found that viral expression was inhibited 5-10 fold by interferon as measured by 1) particle counting by EM; 2) incorporation of ^{3}H uridine into virion RNA; and 3) assays of virion-associated RNA-dependent DNA polymerase (RDDP). Levels of intracellular RDDP activity and viral antigen on cell membranes (measured by immunofluorescence) were not correspondingly depressed, suggesting that interferon acts in part by blocking viral assembly and/or release from the cell membrane.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CB 00517-36 LP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pathological Technology Section

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.J. Coolidge	Supv. Bio. Lab. Tech. (Micro)	LP NCI
OTHER:	L.B. Thomas	Medical Officer	LP NCI
	C.J. Dawe	Medical Officer	LP NCI

COOPERATING UNITS (if any)

FCRC - NCI Project 14

LAB/BRANCH

Laboratory of Pathology

SECTION

Pathological Technology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9	0	9

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

PHS-6040
(Rev. 10-76)

July 1, 1976 - September 30, 1977

Number of investigators	112
Number of pieces of tissue	64,113
Number of bottles of tissue	3,602
Number of blocks cut	36,539
Number of blocks cut serially	1,510
Number of frozen blocks cut	232
Number of blocks recut	1,554
Number of slides stained H & E	54,816
Number of slides stained special	3,817
Number of unstained slides	8,669
Number of slides, H & E, special and unstained	67,302

SUMMARY REPORT

TUMOR IMMUNOLOGY PROGRAM

Fiscal Year 1977

An immunology program has been designed which has as its goals (1) the use of immunologic techniques to detect agents etiologic for cancers, (2) the prevention of cancer by immunologic manipulations, (3) the use of immunologic techniques to improve the diagnosis of cancer, and (4) the manipulation of the immune system of patients with established cancer so that increased survival is achieved.

The program has four operational components (designated Immunobiology, Immunodiagnosis, Immunology of Cancer Cause and Prevention, and Immunotherapy) and each program area has a number of currently funded projects.

A. IMMUNOBIOLOGY

The Immunobiology Program area supports research in immunology that is, or will be, of relevance to tumor immunology.

Acquiring an understanding of the nature of cell surfaces is an area of great importance in studies of the immune system, or tumor cells, and of the interaction of the immune system with tumor cells. Transformed cells appear to have higher rates of lateral diffusion in the membrane than do normal cells (Edidin). Certain types of transformed cells are not plasminogen-dependent (Kiehn). Other aspects of the surface and characterization of transformed cells have been studied (Wallach, Hakomori, Rifkind) including the relationship of cell surface glycoprotein release and metastatic potential (Nicolson) and the mechanism of cell-cell recognition and adhesion (Roseman).

Obtaining detailed information about cell surface protein structure requires new, more sensitive techniques for amino acids sequencing and such techniques have been developed and refined (Smithies, Bennett). Model membranes in the form of liposomes have been used to assess the way in which histocompatibility antigens serve as the locus for immune attack on membranes (Sanderson).

The nature of cellular and humoral immune responses to tumors has been investigated. Autologous tumor cells stimulate proliferation of lymphocytes in 10-30% of patients with selected tumors (Klein). Cell-mediated immunity directed against EBV antigens develops slowly in patients with infectious mononucleosis and may be responsible for terminating the lymphoproliferative aspects of this disease (Stevens). Serologic responses of patients with sarcoma to their own tumors have been detected and then attempts made to define the cell surface antigens responsible (Hirshaut). The nature of the immunoglobulin coating tumor cells in vivo and the

functional role of Fc receptors on tumor cells have also been studied (Witz). Additional cell surface antigens have been found on normal murine T and B cells and on certain lymphoid tumors (McKenzie).

Results of previous studies have suggested that spontaneous tumors may be less immunogenic than those tumors induced for use in the laboratory. Several investigators are attempting to determine whether there is a relationship between antigenicity, degree of spontaneity, and the nature of the immune response to tumors (Embleton, Bartlett, Marquet).

Analysis of the role of lymphocyte subpopulations and macrophages in immunity against tumors has been carried out (Shen, Nossal, Nelson). The types of cells actually involved in the sites of tumor regression have been analyzed (Russell, Kreider), and similar analyses have been performed in a model system utilizing allografts (Hayry). The functional capacity of "natural" killer cells has been explored (Klein, Klein) and a major role for these cells in immune reactions to lymphoid tumors postulated (Wigzell).

Attempts have been made to learn more about the role of suppressor cells in tumor responses (Cerny, Gershon). Circulating immunosuppressive factors have also been studied (Plate).

It appeared that many tumors are able to grow in the presence of an immune response and mechanisms, other than suppression, that make this possible have been investigated. Enhancing factors have been described (Cohen), as well as factors that may reduce lymphocyte recruitment (Lance). Detailed studies of the evolution of immune responses to tumors are being carried out in systems with a significant preleukemic state (Haran-Ghera).

Since T cells play a major role in cytotoxic, as well as suppressor responses, it is important to define the receptor on T cell surfaces that provides specific antigen recognition. Attempts to identify and isolate T cell receptors from normal cells (Maurer) and tumor cells (Roholt, Richards) have been carried out but not much progress has been made. Analysis of the specificity of these receptors suggests that they utilize molecules related to the variable regions of antibody heavy chains (Makela). The possible presence of a transfer factor-like molecule in mouse immune reactions is being investigated (Miller).

The mechanisms by which human K cells lyse antibody-coated tumor cells include drug sensitive steps which operate at the level of the binding of K cells to target cells or other steps which occur at stages following cell interactions (Henney).

A variety of approaches are being taken in an attempt to specifically suppress or enhance aspects of the immune response to tumors (Rowley, Stout, Borel, Schwab, Edwards).

Mechanisms controlling differentiation of macrophages (Metcalf) and lymphoid cells (Toivanen, Basch, Hayry) are being studied. The chemical structure of thymopoietin has been obtained and chemical synthesis of active fragments achieved (Goldstein). Functional activities of lymphocyte activating factor have been further explored (Waksman).

Investigations in many areas of the immunology of cancer are facilitated by projects that generally support the program. Certain types of tumor cell lines are categorized and made available (Cohn) and relatively rare strains of mice, particularly useful for these types of research, are developed and made available (Cherry, McDevitt, Shreffler). Definition of factors in serum that support in vitro cell growth has been studied and the availability of supportive serum has been improved (Mishell). Other supportive projects include attempts to develop automated HLA typing (Grumet), electrophoretic techniques for separating cells (Catsimpoolas), and better techniques for preparing antisera specific for lymphocyte subclasses (Byfield).

B. IMMUNODIAGNOSIS

The fundamental goal of the Immunodiagnosis Program is to develop and evaluate tests which will be useful for the detection and diagnosis of cancer, and for indicating the prognosis of cancer patients and detecting early recurrences of disease. To accomplish this goal, a multifaceted program has been developed. It includes (1) the development and maintenance of resources to support research in immunodiagnosis and to rapidly evaluate the possible clinical usefulness of tests which appear to be able to discriminate cancer from other conditions; (2) the support of research on circulating tumor antigens, with development of sensitive methods for their detection; and (3) the support of research on immune responses to tumor antigens, which might have diagnostic applications.

In the resources area, the maintenance of a serum bank (Schutt) has been invaluable for the rapid evaluation of potentially useful immunodiagnostic and other serum diagnostic tests. During the past year, the availability of coded serum panels has been widely advertised to investigators who had preliminary indications of a useful test. During this period, 45 panels of sera have been sent to investigators in the United States and abroad. It has been possible to accommodate most requests within 2-3 weeks, and after receipt of test results, to analyze the correlation with clinical diagnosis and extent of disease. Much assistance with the statistical analysis has been provided by Dr. Mitchell Gail, Biometry Branch, DCCP, NCI, and by Mr. Michael Stump and his associates in the ADP Management Section, Biometry Branch, DCCP, NCI. This mechanism has allowed us to identify several tests which can significantly discriminate sera of cancer patients from those of normals and of benign disease controls. The results of some of some of these successful tests are summarized below:

Investigator	Assay	Action Taken After Analysis
Linder	Serum copper and ceruloplasmin	Contract initiated
Cooper	Prostatic acid phosphatase	Contract approved

Bucovaz	B protein (non-immunologic)	Referred to Diagnostic Research Advisory Group for possible support
Larson	Isoenzyme alkaline phosphatase (non-immunologic)	Referred to Diagnostic Research Advisory Group for possible support
Hunter	Pancreatic oncofetal antigen	Information given to Diagnostic Research Advisory Group under which this work has been supported
Edgington	CEA-S	Current contract. Continuing comparison of CEA-S with CEA data from other contracts
Marchesi	New GI cancer antigen	Expired contract. Encouraging results communicated to investigators
Theofilopoulos	Antigen-antibody complexes	Current contract
Rossen	Antigen-antibody complexes	Response to RFP invited
Hoch	DNA binding protein	Contract proposal received
Ibrahim	Herpes virus antigen	Response to RFP invited
Kato	Cervical cancer antigen	Response to RFP invited
Rostenberg	Serum glycoproteins	Response to RFP invited
Brown	Nucleosides (non-immunologic)	Referred to Diagnostic Research Advisory Group
Mori	Ferritin	Response to RFP invited
Weltman	Immunoenzymatic assay for CEA	Response to RFP invited
Reddi	Ribonuclease (non-immunologic)	Referred to Diagnostic Research Advisory Group for possible support
Tatarinova	Pregnancy beta-globulin	Information communicated to US-USSR Cooperative Program

In all of the other studies, the data analysis is not yet complete or the results failed to demonstrate significant discrimination between cancer sera and controls. The latter type of information has been very helpful in assessing the validity of a variety of tests, including some which have been reported in the research literature as useful diagnostic tests.

In anticipation of the identification of tests which appear promising for detection of cancer in a high risk population, the Committee on Cancer Immunodiagnosis initiated in 1973 two contracts for the collection of serial serum specimens and clinical information from high risk, screened populations (Najarian, Altschuler). These projects will be completed in the next 1-2 years and the collected sera will be available for rapid evaluation of very promising screening tests.

The other project in the resources area involves the maintenance of a large bank of well-characterized tissue culture cell lines derived from human cancers. The purpose of this contract is to provide to investigators engaged in diagnostic research cell lines which may be used as standard sources of tumor antigens (Fogh). The availability of the cultured cell lines has been widely advertised to investigators in cancer diagnosis research, and a large number of cell lines have been distributed.

Among a large number of projects related to circulating tumor antigens, there are several devoted to studies on the clinical usefulness of CEA and related antigens. CEA is the one immunodiagnostic test which is licensed for general use and is thus widely used. However, there is still insufficient information as to its value for various clinical applications. Therefore, the Immunodiagnosis Program has two contracts, one designed to determine the usefulness of CEA as an adjunct in the differential diagnosis of gastrointestinal cancer (Hersh) and the other for monitoring cancer patients for prognosis and for early detection of recurrent disease (Chu). Within the next year, these projects should provide important new information on the sensitivity and specificity of CEA for these purposes. Since it has been clear for some time that the current assay for CEA does not have optimal sensitivity or specificity, there have been two projects (Reisfeld, Nakamura) devoted to immunochemical studies of CEA and attempts to develop more specific reagents. The work under one of these (Reisfeld) involves the comparison of the usefulness of a variant of CEA, termed CEA-S, with the usual assays for CEA. The data obtained thus far indicate that the CEA-S assay has substantially greater specificity for GI cancer and has similar sensitivity as the assays for CEA.

Assays for alpha-fetoprotein and human chorionic gonadotrophin appear to be quite useful for diagnosis and monitoring of non-seminomatous testicular carcinomas. The Immunodiagnosis Program has supported one project (Moore), which is just being completed, to determine more precisely the sensitivity and specificity of these assays for the initial diagnosis of testicular cancer, and also for longitudinal monitoring of patients with such tumors. The data indicate that these assays are highly specific, with no false

positives, but they fail to detect a portion of the cancers. Therefore, it does not appear that these assays can replace orchiectomy as the definitive method for differential diagnosis.

Fetal sulfoglycoprotein in gastric juice has been shown to have promise for screening for gastric cancer. The Program is supporting a project (Hakkinen) to determine more precisely the value of this test for early detection of gastric cancer, and to develop a more sensitive assay.

Patients with cancer may produce antibodies directed against circulating tumor antigens. Sensitive methods for detection of circulating antigen-antibody complexes have been developed and these procedures are being used to explore their value as diagnostic tests and as methods for detection of new tumor antigens (Theofilopoulos, Schrohenloher, Burger). In the studies performed thus far, it is clear that a substantial proportion of cancer patients have elevated levels of complexes and that tumor-associated antigens can be identified in some complexes.

The Immunodiagnosis Program has supported a number of projects for development of new human tumor-associated antigens which might be useful in immunodiagnosis. An emphasis has been placed on studies of antigens associated with the more common tumor types: lung cancer (Veltri, Weimer), gastrointestinal cancer (Alpert, Marchesi, Saravis), cervical cancer (Alexander), ovarian cancer (Lloyd), and acute leukemia (Humphreys). Preliminary evaluation of one of these antigens with a coded serum panel has yielded quite encouraging results (Marchesi). In addition to these organ-related projects, the Program is also supporting one project (Linder) for measurement of serum copper and ceruloplasmin. These assays were first shown to be able to discriminate cancer sera from controls in a coded panel; and since initiation of a contract, emphasis has been placed on the usefulness of these tests for diagnosis of lung and breast cancer.

Related to efforts on circulating tumor antigens, the Program is also supporting a project on an experimental animal model for circulating tumor antigens (Osborne) and one for improved methods for chemical characterization of the antigens (Bahl).

Detection of antibodies to tumor-associated antigens has great potential for early detection of tumors. The Immunodiagnosis Program has supported two projects utilizing animal models to develop more sensitive procedures for antibody detection (Manson, Stutman). The latter project, studying antibody-dependent cell-mediated cytotoxicity, has provided much new information about this phenomenon and may be able to detect early mouse mammary tumors. Immunofluorescent procedures have been widely used for the study of antibodies to human tumor antigens, and two projects on the usefulness of these tests for detection of specific antibodies to autologous tumor antigens have been supported. One of these projects (Friou) has detected a high proportion of such antibodies whereas the other (Paronetto) has detected very few. Efforts are being made, including exchange of materials, to determine the apparent differences in results. One additional project (Chiu) involves the study of antibodies to

chromosomal non-histone proteins. The data obtained thus far indicate that specific antibodies to organ-associated tumor antigens can be identified.

The last major research area being supported by the Immunodiagnosis Program is concerned with studies of cell-mediated immunity to human tumor antigens. To facilitate studies in this area, it has been thought advisable to develop better methods for identification and isolation of effector cells that might be involved in these reactions. Two projects have focused on methods for separating subpopulations of blood lymphocytes (Wigzell, Schlossman). Both of these projects have provided much important new information on markers for lymphocyte subpopulations and on methods for their physical separation. Another project (Talmage) has been devoted to isolation of peripheral blood monocytes in high yield and purity, and this has been quite successful. In order to standardize the assays for cellular immunity and to facilitate exchange of cells among laboratories, cryopreservation of functionally active cells is quite important and one project (Schlossman) has developed practical methods for freezing of human lymphocytes.

Assays for delayed cutaneous hypersensitivity reactions to tumor antigens, and related in vitro assays, have proven useful to identify and isolate human tumor-associated antigens. There are three projects (Wright, Reisfeld, Malley) concerned with this and their main objective is to make antibodies against tumor antigens involved in these cellular reactions, in an attempt to develop more sensitive and practical immunodiagnostic assays, especially radioimmunoassays. Two of these projects have had some success in preparing heteroantibodies to isolated, melanoma-associated antigens and the third has detected kidney cancer-associated antigens.

Two projects in this area have focused on improvement in methods for detection of cell-mediated immunity. One (Henney) has been restricted to mouse tumor models and the other (Harrington) has progressed from a mouse model to studies with human tumors. The method under study in the latter project, an agarose microdroplet method for detection of leukocyte migration inhibition, appears quite promising for large scale application to the study of human cell-mediated immunity to tumor antigens.

A procedure which has been claimed by several groups to be highly sensitive and specific for detection of cellular immunity to a common human tumor antigen is that of macrophage electrophoretic mobility. A contract has been awarded to one of the original proponents of the test (Pritchard) to determine its ability to discriminate cancer patients from controls in a study of coded blood specimens provided by a separate, local institution. Another project (Weiss) is in progress to determine whether the test can be duplicated in a different laboratory in the United States and this group is also testing coded blood specimens. The results of the tests on the coded specimens by the former project have just been received, and they clearly indicate that the test has serious problems with both sensitivity and specificity and the earlier results by this group, which were received with much enthusiasm and which were widely publicized, were not confirmed.

In summary, the most important accomplishment of the Immunodiagnosis Program has been the development or identification of several very promising immunodiagnostic tests for cancer, which have been validated by their ability to discriminate between specimens of cancer patients and controls. Furthermore, these objective methods for evaluation of tests have provided rapid indications that some tests, which had previously been thought to be valuable, could not adequately discriminate.

C. IMMUNOLOGY OF CANCER CAUSE AND PREVENTION

The goals of the Immunology of Cancer Cause and Prevention Program are to develop immunologic methods that may be useful in experimental studies on cancer cause and prevention. Means are being sought to (1) detect carcinogens in the environment and in body fluids and organs, (2) uncover and correct possible immunologic defects accompanying carcinogenesis and (3) inhibit tumorigenesis before the development of recognizable malignancy.

1. Detect carcinogens in the environment and in body fluids and organs: It was observed that the ability of macrophages to synthesize certain complement components in vitro was markedly reduced if they were obtained from guinea pigs treated with certain chemical carcinogens (Colten). This work is now in its terminal year. These findings are being exploited as a possible means of detecting environmental chemical carcinogens (Strunk). Attempts have been made to develop cell-mediated immunologic methods for the detection of exposure to carcinogens (Thor). Formidable technical problems indicate that further development of this approach through the contract program must await basic advances; the contract has been terminated.

Radioimmunoassays have been developed for the detection of a variety of chemical carcinogens, nicotine and cotinine, insecticides, and chemotherapeutic agents (Van Vunakis). The feasibility of detecting picomoles of these compounds in body fluids has been established. An RFP has been advertised to exploit these techniques but the response has been disappointing. Attempts will be made to study the applicability of the methods developed within the existing contract.

2. Uncover and correct possible immunologic defects accompanying carcinogenesis: A study, based on the observation that some chemical carcinogens are also contact allergens, is in its terminal year (Pomerance). Guinea pigs were first made immunologically unresponsive to chemical carcinogens and their susceptibility to tumorigenesis by the corresponding carcinogen was compared to that of animals sensitized to the agent. These and untreated animals did not differ significantly in the rate and extent of chemical carcinogen-induced tumor formation. Intravenously administered dimethylbenzanthracene produced breast tumors which to date, more than a year later, have not metastasized.

Chemotaxis of human peripheral blood monocytes was depressed in a significant number of cancer patients (Synderman). Immunotherapy with BCG or surgical removal of the neoplasm reduced the percentage of patients

with depressed chemotaxis. The best prognosis was in patients who had normal chemotaxis prior to therapy. This contract is in its terminal year. Two projects (Zwilling, Osler) are devoted to studies of the effects of chronic administration of low doses of chemical carcinogens to animals on various aspects of immunity. So far immune reactivity, based on the results of a variety of tests, has been unchanged or only minimally affected. Changes that have been observed occur when neoplasia is evident. Results so far, therefore, indicate that low doses of carcinogens that nevertheless produce tumors in a small proportion of animals, cause no detectable immunologic defects.

3. Inhibit tumorigenesis prophylactically: A group of projects (Goren, Gensler, Andrews, Clark) is directed toward the use of BCG or its subfractions as a means of interrupting carcinogenesis before the development of cancer.

a. It is known that trehalose dimycolate, a fairly well-defined fraction of BCG, is partly responsible for the anti-tumor action of BCG. This compound, as well as related structures of mycobacteria that have been isolated and characterized, will be supplied to various investigators who wish to test them for immunoprophylactic anticancer activity (Goren).

b. An attempt is being made to totally synthesize trehalose dimycolate (Gensler).

c. Primary malignant melanoma, pigmented and metastatic, is being induced by dimethylbenzanthracene in allogeneic guinea pigs. Attempts are being made to interfere with tumorigenesis by the injection of BCG into pigmented premalignant lesions. This activity is being terminated (Clark).

d. No progress to report because containment facilities are not yet completed at this contract site (Andrews). The plan is to try to interrupt the development of chemical carcinogen-induced colon cancer in guinea pigs by the injection of BCG into premalignant lesions.

4. Major accomplishment: A major accomplishment has been the successful and routine production of pigmented, metastatic primary autochthonous malignant melanoma in allogeneic guinea pigs. Immunoprophylactic studies of this and other experimental primary malignancies should provide information with greater relevance to human neoplasia than those carried out using inbred animals with transplanted, induced, often non-metastasizing tumor. A logical extension of these studies will be investigations of naturally occurring, agnogenic, metastatis malignancies in randomly-breeding mammals.

D. IMMUNOTHERAPY

The goals of the Immunotherapy Program are to (1) identify immunologically active agents that lead to cure, regression or stabilization of established tumors; (2) determine the mechanisms whereby such agents work; and (3) assess the effectiveness of these agents in patients with cancer.

Evaluation of imunologically active agents is performed in animals bearing tumors that have some resemblance to human tumors, or that are already known to be responsive to immunologic manipulations. Once suitable tumor systems are identified, agents known to be immunologically active can be optimized and/or compared for relative efficacy, and agents that are suspected of being active can be tested. BCG, MER, C. parvum and tumor cell vaccines have been evaluated with several mouse tumors, one rat and one guinea pig tumor (Bartlett, Kollmorgen) and differential activity was demonstrated in different systems. Both BCG and C. parvum appear to be more effective in suppressing tumor growth when they are in direct contact with rat tumors than when they are given systemically (Baldwin). BCG and C. parvum cause only minimal suppression of a guinea pig leukemia under the conditions evaluated (Murphy). Combination of systemic chemotherapy with intratumoral injection of C. parvum led to almost 100% total permanent regression of mouse mammary tumors, while the same chemotherapy with systemic C. parvum was ineffective (Fisher).

Further studies of the requirements for activity of BCG (Collins), MER (Weiss), neuraminidase-treated tumor cells (Kollmorgen, Gershon), and immunopotentiators (Kaplan) have been carried out.

Inbred chickens provide a potentially useful model for immunotherapy because of the unique manipulability of the B cell as well as the T-cell systems. Studies with carcinogen-induced fibrosarcomas have been initiated (Lerman). Leukemias and lymphomas occurring spontaneously in outbred cats provide another model of interest and studies of passive and active immunotherapy will be carried out (Essex).

Studies of newer approaches to immunotherapy have been supported. Glycosidases are being used to modify tumor cell membranes and assess effects on membrane immunogenicity (Pincus). Tumor cells have shown antigenic modifications after being exposed to chemotherapeutic agents (Bonmasser, Arai). In addition individual tumors have been shown to be heterogeneous with regard to cell surface carbohydrate and the effectiveness of sub-populations in immunotherapy experiments appeared to be related to the cell surface carbohydrates expressed (Killion).

Immunologically competent cells have been sensitized against tumor cells in vitro and then assessed for anti-tumor effects (Palmer, Kedar, Kaplan). Macrophages, which may have non-specific anti-tumor cell activity, have been activated in vitro and tested for cytotoxic activity in vivo (Piessens).

Other new approaches being investigated include conjugating chemotherapeutic agents to anti-tumor antibodies to determine whether this improves chemo-therapeutic effects (Arnon), inducing thyroiditis in rats to see whether

the induction of immunity to an organ can lead to destruction of a tumor originating in that organ (LoGerfo), studying the ability of tumor necrosis factor to destroy established murine tumors in vivo (Old), probing the effectiveness of amphoterecin B as an immunostimulant (Little), attempting (unsuccessfully) to develop evidence for the usefulness of immune RNA in the treatment of rodent tumors (Deckers), attempting further studies of immune RNA and transfer factor in primates (Eichberg, Baram) and of transfer factor in man (LoBuglio), and determining whether lymphokine preparations will induce regressions of animal tumors (Papermaster).

Macrophages appear to play an important role on both the afferent and efferent sides of many immune interactions. Comparisons have been made of the ability of various macrophage activators to cause macrophages to inhibit tumor cell growth (Krahenbuhl). The functional characteristics of macrophages from normal and tumor bearing animals have been compared (Schmidtke) and assays for human monocyte function have been developed and applied to studies of some cancer patients (Schmidtke, David, King).

Antigen-antibody complexes and tumor antigens in the circulation may be involved in the generation and/or suppression of immune responses to tumors. The concentration of these substances in the circulation has been studied, and the kinetics of production and effects of immunosuppressive treatments analyzed (McKhann, Feldman).

The clinical immunotherapy program has included a number of trials designed to determine maximum tolerable doses and evidence for effect on the immune system of agents that are supposed to be immunologically active. The agents investigated are C. parvum and levamisole (Hirshaut), various BCG preparations (O'Connell, Klein), mycobacterium phlei cell walls (Vosika) and different types of active specific immunotherapy (Zighelboim, Oettgen).

Clinical trials to determine effectiveness of immunotherapeutic agents have yielded interesting results in lung cancer. The introduction of living BCG into the pleural space after resection of lung cancer has significantly increased the disease free interval and survival of patients with stage I disease when compared to randomly selected control patients treated with surgery alone (McKneally). Other immunotherapeutic trials in patients rendered free of disease by surgery include the use of a soluble, partially purified extracts of lung cancer membranes, mixed with complete Freund's adjuvant (Takita), the use of MER postoperatively in patients with squamous cell carcinoma of the lung (Cortes), treatment with levamisole in patients postoperative for squamous cell cancer of the head and neck (Wanebo), and specific or nonspecific immunotherapy as an adjuvant to chemotherapy in sarcoma (Eilber) or alone after surgery in melanoma (Morton).

Vibrio cholera neuraminidase treated blast cells are being evaluated for therapeutic efficacy in adult acute myelogenous leukemia (Holland) and "unblocking" plasma effects are being assessed in melanoma (Hellstrom).

Patients with more advanced stages of cancer are being treated with many of the same agents (Hersh, Simmons, Fahey)., Prolongation of survival has been demonstrated in these patients with breast cancer who have tumor regression when treated with chemotherapy and C. parvum (Pinsky).

Radiotherapy apparently causes strong immunosuppression. Studies are being carried out to document and analyze such suppression and to study the ability of immunostimulating agents to counter such suppression (Wara, McLaren). Additional studies of immunologic responsivensss are being performed in patients with osteosarcoma (Smith).

Work that is potentially useful to the field of immunotherapy has also been supported. A fully viable preparation of BCG has been produced and distributed (Miller), an attempt has been made to define "inbred" strains of rabbits whose sera might be particularly useful as a complement source for tumor immunology serology (Cherry), and development of a new, standardized skin test device has been supported (Hein).

The outstanding accomplishment in work supported by this program in the past year has been the study of McKneally indicating that intrapleural BCG can prolong survival of patients with stage I lung cancer.

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Robert Stout
Name/Address Brandeis University
Performing Organization: Rosenstiel Research Center
Waltham, MA

Contract Number: NO1-CB-64034
Starting Date: 6/15/76 Expiration Date: 12/31/77

Goal: To develop a system for stimulation of isolated amplifier T cells so as to allow amplification of cytotoxic responses to syngeneic cells which bear tumor-specific antigens but no allogeneic Ia antigens.

Approach: The approach entails a systematic study of the effect of mixed lymphocyte and Con A-stimulated subpopulations on the cytotoxic responses of unstimulated subpopulations of T cells to allogeneic and syngeneic tumor cells. The subpopulations of T cells will be detected by the presence of surface Ly or FcR determinants and isolated on a Fluorescence Activated Cell Sorter.

Progress: Equipment required for this project was to be obtained with monies provided by NIH grant 1 RO1 AI 13806-01, which became available 2/1/77. Required reagents and animals have been prepared or secured (including anti-Ly sera). The cell sorter is functional and manned by a competent operator. Test separations have been successfully run. The in vitro culture and assay systems for Con A and mixed lymphocyte stimulation and cytotoxic lympholytic reactions have already been successfully established. In short, in the past two months all work preparative for active experimentation has been completed.

Significance to Cancer Research: NCP Objective 6 Approach 4.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Dr. Richard A. Rifkind
Name/Address Columbia University
Performing Organization: College of Physicians and Surgeons
New York, NY

Contract Number: NO1-CB-44008
Starting Date: 5/1/74 Expiration Date: 4/30/77

Goal: To determine the role of cell surface components in the initiation of erythropoietic differentiation in murine erythroleukemia cells induced by a variety of chemical agents.

Approach: Three approaches: (1) structural, including thin-section and freeze-fracture electronmicroscopy; (2) incorporation of labeled inducing agents into membrane components; (3) effect of inducing agents upon cell membrane-mediated functions, in particular, cyclic nucleotide accumulation.

Progress: Extensive exploitation of morphological techniques has to date failed to reveal uniquely characteristic changes which can be attributed to early expression of induced differentiation. Nevertheless, it is apparent that a very significant fraction of labeled inducing agent becomes associated with plasma membrane constituents and the nature of this association is under investigation. Furthermore, one of the earliest recognizable manifestations of inducer effect on inducer-sensitive cells is the accumulation of cyclic AMP. This is accompanied by an arrest of the cells in G_1 of the cell cycle, suggesting a possible relationship between a cell surface and a cell cycle effect of inducers.

Significance to Cancer Research: Inducing chemicals not only promote differentiation but decrease tumorigenicity in susceptible host mice. Therefore, the mechanism of induced differentiation may prove of significance in the design of novel therapeutic agents for selected neoplastic diseases.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. Sen-itiroh Hakomori
Name/Address Fred Hutchinson Cancer Research Center
Performing Organization: Seattle, WA

Contract Number: NO1-CB-64067
Starting Date: 6/1/76 Expiration Date: 5/31/77

Goal: (1) Isolation and characterization of "galactoprotein a" and "ceramide X." (2) Isolation and characterization of "pseudo A antigen" in human tumor.

Approach: "Galactoprotein a" (or LETS protein) is present only on normal cell surface and deleted in various transformed cells. Isolation and characterization of this glycoprotein have been studied. "Ceramide X" is a unique glycolipid label only on the surface of NILpy tumor cell but not on NIL cell. The presence of glycolipid was revealed by surface labeling with galactose oxidase and [^{3}H]borohydride. "Pseudo A antigen" is an A-like antigen appearing in human tumors from blood group O or B. Isolation and characterization of this antigen were studied.

Progress: (1)"Galactoprotein a" was isolated in essentially pure form from hamster NIL cells by sequential solubilization by (i) sucrose-ATP, (ii) zwitterionic detergent and Tris, and (iii) Urea-Tris buffer followed by affinity chromatography on Ricinus communis Sepharose column. Amino acid composition was determined. Significantly, "galactoprotein a" associates with actin-like material. (2) "Ceramide X" was identified as βGal1→4βGlcNAc1 →3βGal1→4βGlc→ceramide. Antibody against this glycolipid was found in NILpy tumors growing in hamsters. (3) "Pseudo A" antigen was identified as a Forssman glycolipid.

Significance to Cancer Research: Discovery and the identification of "ceramide X" and "pseudo A" antigen will open a basis for possible immunotherapy for animals and human cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Jan Cerny
Name/Address Harvard College
Performing Organization: Cambridge, MA

Contract Number: NO1-CB-64035
Starting Date: 6/28/76 Expiration Date: 6/27/77

Goal: The goal is to elucidate the nature, properties, and mode of action of immunosuppressive cells which appear in the lymphoid organs of tumor-bearing mice and which actively inhibit the functions of immunocompetent cells in the organism.

Approach: The principal experiments are done by mixing cells in vitro. Thus, cells from various organs (spleen, lymph nodes, or thymus) of mice with malignant tumors (a leukemia or sarcoma) are added into cultures of normal, syngeneic immunocompetent lymphocytes (i.e., responder cells) which are being stimulated with an antigen. A diminution of the response indicates the presence of active immunosuppressive cells in the given organ of the tumor-bearing donor.

Progress: The experiments during the first year concentrated on mice with lymphoma/leukemia (Moloney). From previous work, it was known that spleens from such mice contained many cells capable of suppressing the immune response of normal lymphocytes to sheep erythrocytes in vitro. During the first year of the contract, it was confirmed that the inhibition requires a cell contact between "suppressors" and "responders" and that a transmission of Moloney virus is not responsible for the inhibition. The most exciting is the finding that a removal of most if not all leukemic cells from the spleen cell suspension does not diminish its suppressive effect; this strongly suggests that these are normal, nontumorous immunosuppressive cells in the leukemic spleen. Also exciting is the finding that the suppressive effect can be abolished by the addition of Ca^{++} into the culture medium.

Significance to Cancer Research: Immunosuppressive cells are now being found also in patients with tumors. It is likely that these cells represent one of the reasons why the organism is unable to mount effective immune response to the tumor as well as to various infections. The mouse model permits the analysis of this problem.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Detection of Antigen-Binding Activity of Transplantable T-Cell Tumors

Principal Investigator: Dr. Oliver A. Roholt
Name/Address Health Research, Inc.
Performing Organization: Roswell Park Memorial Institute
Buffalo, NY

Contract Number: NO1-CB-43924
Starting Date: 6/16/74 Expiration Date: 6/15/77

Goal: To develop a suitable assay for recognizing specific antigen-binding sites on T-cell tumor cells and through this assay to identify the antigen-binding specificity of the antigen-binding receptors on one or more murine T-cell tumor lines.

Approach: To compare the binding, by the cells of a number of T-cell tumor lines, of each of a series of ^{125}I-labeled antigens with that of the binding of a ^{131}I-labeled reference antigen, both radiolabeled antigens of each pair present in the same mixture. The cells and radiolabeled antigen mixture are incubated together in the cold, the cells centrifuged, washed and then the ratio of the bound ^{125}I to the bound ^{131}I determined and compared with the ratio for the initial incubation mixture. An increased level of bound ^{125}I in the case of a particular ^{125}I-labeled antigen indicates binding of that particular antigen.

Progress: The 3-nitro-4-hydroxy-5-iodophenylacetyl- (NIP-) protein conjugate has, in some experiments but not readily repeatable, been found to be bound by the cells of three murine T-cell tumor lines and the 2,4-dinitrophenyl- (DNP-) protein conjugate by two T-cell tumor lines, one of which is also one of the apparent NIP-binders. We have been isolating, by NIP and DNP affinity chromatography of the supernatant of 18-hr cultures of the cells of the above tumor lines, substances which may represent the specific shed receptors and which we are characterizing, as the amounts of materials accumulate, by SDS-gel electrophoresis and by antigenic properties and relationships.

Significance to Cancer Research: The now well-established role of the T cell in regulating the immunological response of an individual, together with knowledge about the T-cell antigen receptor and its function in the initial recognition phase of this regulation, will permit a better exploitation of T-cell function in manipulating cancer immunity.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cell-Mediated Immunity to Epstein-Barr Virus

Principal Investigator: Dr. David Stevens
Name/Address Institute for Medical Research of
Performing Organization: Santa Clara County
San Jose, CA

Contract Number: NO1-CB-53957
Starting Date: 6/16/75 Expiration Date: 6/15/77

Goal: Epstein-Barr virus (EBV) causes infectious mononucleosis (IM), a benign disease characterized by lymphoid proliferation. EBV is also suspected to cause cancer in man. Studies of the immune response to EBV have associated certain patterns of humoral immunity with specific malignancies and subtypes. These appear to be of prognostic value and may give clues to etiology. We proposed to establish assays of cellular immunity (CI) and study their relation to EBV.

Approach: Viral and viral-induced antigens were compared for their potency in stimulating CI. Patients with IM were studied to learn what terminates their cellular proliferation. The CI assays were lymphocyte stimulation (LS) in vitro and lymphocyte production of the cell-killer substance, lymphotoxin (LT). Nonspecific stimulants such as PHA were also studied.

Progress: EBV capsid and soluble viral-induced (S) antigen produced LS significantly greater in seropositive than seronegative healthy donors, in contrast to PHA. In IM, both PHA-induced and spontaneous transformation were depressed. IM patients had antigen-specific LS below seropositive donors initially and low responses for the acute phase of illness when clinical symptoms were present and antibody titers maximal. Specific LS rose to a peak at 3.5-9 weeks when the patients had recovered, most lab findings had returned to normal, and viral and heterophile antibodies had declined. At peak, specific LS in IM exceeded that of seropositive donors, but later declined. LT production was correlated with LS. Spontaneous LT production was especially augmented initially in IM. These results demonstrate specific CI to EBV. They indicate that CI develops slowly in IM and contrasts with the evolution of the clinical events and humoral immunity. CI could thus be the mechanism of terminating lymphoproliferation in IM. IM patients have a transient serum factor blocking LS.

Significance to Cancer Research: The evidence that CI may be important in terminating the lymphoproliferative disorder, IM, could be important in approaches to controlling malignant lymphoproliferative diseases, particularly Burkitt's lymphoma and nasopharyngeal carcinoma.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Production and Distribution of H-2 Recombinant or Mutant Congenic Strains of Mice

Principal Investigator: Dr. Marianna Cherry
Name/Address Jackson Laboratory
Performing Organization: Bar Harbor, ME

Contract Number: NO1-CB-43988
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: (1) To provide quality control for health and genetic purity on specialized stocks of research mice; (2) to explore ways of making these stocks more widely available; and (3) to produce new H-2 recombinant strains of mice.

Approach: (1) Testing for genetic purity by exchange of skin grafts and for animal health by testing within this laboratory and by an outside consultant; (2) expanding research stocks of specified strains of mice on an experimental basis and estimation of demand; and (3) examination of appropriate segregating populations of mice for naturally occurring H-2 recombinants.

Progress: Several lines have been isolated that carry mutant or recombinant genotypes at the mouse H-2 complex, which affects a number of immunological processes, including an (indirect?) effect on development of some types of tumors. Genetic purity of lines in research colony by appropriate skin grafts has been established; all animal health parameters readily testable have also been found satisfactory. To test demand for increased stocks of these specialized animals, their supplies have been expanded and outside investigators were advised of increased supplies. Availability has risen with demand from 200-300 mice/year to 2,000-3,000. An animal was identified with a new and long-sought H-2 recombinant genotype in the mouse colony ($H\text{-}2K^{b}H\text{-}2D^{K}$). Progeny have been obtained from this animal, which is particularly interesting since it derives from an original mating between a high-leukemia strain of mice (AKR/J) and a low-leukemia strain (C57L/J).

Significance to Cancer Research: Increased numbers of animals are now available for research, in good health and defined genetic status, for studies of the effect of the mouse H-2 complex on tumorigenesis. A new H-2 haplotype of a particularly valuable type has been found and propagated.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T Cells

Principal Investigator: Dr. Paul Maurer
Name/Address Jefferson Medical College
Performing Organization: Thomas Jefferson University
Philadelphia, PA

Contract Number: NO1-CB-43999
Starting Date: 5/1/74 Expiration Date: 5/15/77

Goal: To isolate and purify T cells (and B cells) from normal immune responder and nonresponder mice immunized with the polymer GAT^{10}; to determine the purity of the cells, and the specificity of binding to homologous polymers; and to isolate soluble T-cell receptors from mice used in the above studies.

Approach: To immunize mice with GAT^{10}, fractionate the lymphoid cells on nylon wool columns, measure the purity using appropriate T- and B-cell mitogens (PHA and LPS), and then measure the in vitro proliferation response in the presence of ^{3}H-thymidine caused by the homologous, heterologous and non-cross-reacting polymers. Subsequently, similarly prepared cells were disrupted for the isolation and solubilization of plasma membranes. The reaction of the membrane with polymer was followed to isolate the "polymer-specific T-cell receptor" complex.

Progress: During the present contract year the contractor concentrated on solubilizing the plasma membranes, measuring specific antigen interaction with these membranes, and characterization of the physical and immunological properties. Two procedures have been employed for solubilization of membranes: (1) 1.5 M acetic acid - 9 M urea - 0.1% NP-40 mixtures, and (2) NP-40 at concentrations that would not be destructive of biological binding ability (0.03% NP-40 was optimal). Ultracentrifugal techniques in sucrose gradients proved the best to measure the interactions with the putative T-cell receptor. The "antigen-receptor" complex was isolated and characterized. Two main receptor components were found to have sizes of 30,000 and 60,000 daltons. The most recent data indicate that these components bind antigen, do not have classical immunoglobulin structures, and do not have Ia determinants.

Significance to Cancer Research: The study of the immunological specificity and biochemical nature of antigen-binding receptors on T cells may help (a) explain the mechanisms of tumor cell surveillance by activated T cells, and (b) hopefully find application to tumor therapy.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Dr. Michael Edidin
Name/Address Johns Hopkins University
Performing Organization: Baltimore, MD

Contract Number: NO1-CB-43922
Starting Date: 5/1/74 Expiration Date: 4/30/78

Goal: Show differences in the diffusional mobility of surface proteins when comparing transformed and nontransformed cells. Such differences would re-enforce the view that changes in the arrangement of all surfaces are a major factor in malignancy. Factors affecting or changing diffusion rates, if elucidated, are to be used in modifying cell behavior and growth properties.

Approach: Measure lateral diffusion of membrane antigens in a wide range of cells using two techniques: (1) Formation of heterokaryons and analysis of the antigen distribution in the population; and (2) surface fluorescent labeling cells, then bleaching a spot in the label and measuring return of fluorescence to the spot.

Progress: Initially the contractor used a cell population method, formation of heterokaryons, and analysis of their antigen distribution, for measuring protein lateral diffusion in the cell membrane. This method indicates that diffusion in transformed cultured cells is approximately 10 times slower than that expected for free diffusion of protein in lipid. Further work showed that diffusion was yet another order of magnitude slower than expected in heterokaryons made from nontransformed cells. Since then a second method was developed, photobleaching of label on single cells, for measuring lateral diffusion. This method gives values for protein diffusion in good agreement with previous results. It was possible to alter diffusion rates by treating cells to change their transmembrane potentials.

Significance to Cancer Research: Since recent results indicate that membrane diffusion rates may be controlled by altering transmembrane potential, it may be that treated, transformed cells will revert to nontransformed in growth properties. Also, if the difference in diffusion rates between transformed and nontransformed cells holds consistently, it may be useful as a diagnostic aid.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $65,094

CONTRACT RESEARCH SUMMARY

Title: Mechanisms for Cell-Mediated Destruction of Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing Organization: Baltimore, MD

Contract Number: NO1-CB-43932
Starting Date: 5/1/74 Expiration Date: 4/30/78

Goal: This contract is aimed at elucidating the mechanism by which human peripheral lymphocytes (K cells) are capable of lysing antibody-coated tumor cells.

Approach: The destruction, by peripheral lymphocytes, of a polyploid human liver cell line (Chang cells) coated with rabbit or human IgG antibody has been followed in a short-term ^{51}Cr release assay. A variety of drugs known to affect lymphocyte metabolism have been studied for their effects on cytotoxicity, hoping thereby to provide insight into the mechanism of cytolysis.

Progress: The assay system developed has enabled a reproducible, quantitative assessment of the cytotoxic capacity of human peripheral lymphocytes and has provided data that indicate that functional K cells represent of the order of 4% of human peripheral lymphocytes. It has been determined that drugs which inhibit cytolysis in vitro do so either by acting (i) at the level of interaction between K-cell Fc receptors and IgG-coated target cells (as do the cytochalasins A and B), or (ii) at a stage which follows such cell-cell interaction (e.g., the cAMP "active" drugs: prostaglandin E_1, isoproterenol).

A binding assay has been developed which allows isolation of K cells in high yield and with a lytic activity 5-10 times greater on a cell basis than unfractionated lymphocytes.

Significance to Cancer Research: NCP Objective 6 Approach 4

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $57,090

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. Saul Roseman
Name/Address Johns Hopkins University
Performing Organization: Baltimore, MD

Contract Number: NO1-CB-43985
Starting Date: 5/16/74 Expiration Date: 5/15/77

Goal: To gain basic information on the biochemical and morphological mechanisms involved in intercellular adhesion.

Approach: Several approaches were used, including the following: (a) Transmission and scanning electron microscopy in conjunction with autoradiography to examine cell-cell junctions formed shortly after cells had come into contact and adhered to one another, and (b) the synthesis of insoluble polymers derivatized with sugars. Cells were then exposed to the derivatives to determine whether they recognized and adhered to specific carbohydrates.

Progress: The process of intercellular adhesion was shown to be multi-step within the first few minutes, not a "lock-and-key" type mechanism. Initial work with solid matrices derivatized with sugars showed that SV40 transformed 3T3 cells specifically adhered to galactose-derivatized Sephadex beads. These beads were synthesized by the cyanogen bromide procedure. The cyanogen bromide activation method is extremely troublesome in that it yields cationic charge on the beads which can give artifacts when working with cells or using affinity chromatography. Procedures were developed for measuring and minimizing this charge. Now only polyacrylamide and polystyrene matrices are used. These offer major advantages, either for work with cells or for affinity chromatography. For example, preliminary results indicate that such derivatives may prove useful in isolating pure populations of killer T lymphocytes. They have also been used to study the adhesive properties of hepatocytes.

Significance to Cancer Research: Cell-cell recognition and adhesion are of pivotal importance in phenomena such as metastasis. If the biochemical basis for intercellular adhesion can be determined, it may lead to a rational approach to the prevention of metastases.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Effector Mechanisms in Tumor-Associated Immune Reactions

Principal Investigator: Dr. Eva Klein
Name/Address Karolinska Institute
Performing Organization: Stockholm, Sweden

Contract Number: NO1-CB-33870
Starting Date: 6/25/73 Expiration Date: 7/24/76

Goal: Relative role of humoral antibodies and different lymphocyte subclasses in tumor immunity.

Approach: Studies on experimental and human tumor cell systems in vitro and some mouse tumor systems in vivo.

Progress: The natural killer (NK) cell activity, displayed by a non-T, non-B lymphocyte fraction of certain mouse strains against a variety of murine target cells, was found to be under genetic control, with a relatively strong H-2 linked factor. Antibody formation against virally determined, tumor-associated antigens on the surface of some target lines was also under genetic control, but independent of the system that influences the NK reactivity. In chemically induced rodent gliomas and sarcomas, a clear distinction was obtained between non-T cell-mediated, nonspecific killing and T cell-mediated, specific killing. In the human systems, tumor infiltrating, EBV-specific killer T cells could be isolated from Burkitt lymphomas and nasopharyngeal carcinomas. This killing was directed against the EBV-determined LYDMA antigen. A different type of EBV-specific killing, directed against EBV-MA, could be obtained in an in vitro ADLC system. A difference could be demonstrated in the tumorigenic potential of EBV-transformed lymphoblastoid lines of normal origin, as long as they were diploid, and EBV-carrying Burkitt lymphoma lines; the latter but not the former could grow regularly in nude mice. The K-562 line could be characterized as the first human line of myeloid origin, continuously growing in vitro. A new enzymatic marker (N-Apase) was found in both mouse and human leukemia cells and derived lines.

Significance to Cancer research: At this time it is essential to distinguish between in vivo relevant tumor-associated immune reactions with a rejection potential and irrelevant or nonspecific in vitro lymphocytotoxicity, both in experimental and human systems. Our endeavors were directed toward such a distinction on selected experimental and human systems.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens and Corresponding Antibodies

Principal Investigator: Dr. Eva Klein
Name/Address Karolinska Institute
Performing Organization: Stockholm, Sweden

Contract Number: NO1-CB-43921
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: Search for tumor-associated antigens and antibodies in human sarcoma, carcinoma, and glioma patients.

Approach: Stimulation of autologous lymphocyte by tumor biopsy cells in vitro (MLTI-reaction).

Progress: Stimulation of blood lymphocytes by in vitro exposure to autologous biopsy cells has been recorded in 10-30% of the patients, depending on the tumor system investigated. This stimulation did not occur if autologous serum was present during the period of interaction or if the stimulator cells were preincubated in autologous serum. There was a certain cross reactivity between patients of the same tumor type, in blocking experiments, but the specificity patterns require further study. Tumor-associated lymphocyte stimulation could be obtained with tumor extracts, in addition to live tumor cells. There was a certain inverted correlation between the ability of a given tumor biopsy to stimulate and its immunoglobulin content. Changes in lymphocyte stimulability and serum blocking activity were recorded in horizontal patient studies.

Significance to Cancer Research: The MLTI method is one of the few that have given potentially meaningful information about tumor-associated immune reactivity in autologous human systems. The exploration of the antigenic specificities involved and the possible clinical correlations are of great importance for the understanding of human tumor-associated immune reactions.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Eva Klein
Name/Address Karolinska Institute
Performing Organization: Stockholm, Sweden

Contract Number: NO1-CB-64023
Starting Date: 6/28/76 Expiration Date: 6/27/77

Goal: This study focuses on characterizing the naturally occurring cytotoxic cells (NK cells) in mice and man, partly to assess their significance and partly to develop methods to remove them, in order to reveal possibly specific reactions that are otherwise masked by them.

Approach: Lymphocyte fractionation and lymphocytotoxicity in human systems; the same in combination with in vivo rejection tests in mouse systems.

Progress: NK cell activity vs. killing by sensitized T cells showed target-cell type dependent variations in a comparison of cells carrying the same virally determined surface antigen complex. During tumor growth, NK vs. T cell-mediated reactions could change in the opposite direction. T cell-mediated killing obeyed the rules of H-2 associated syngeneic restriction, whereas NK-mediated killing did not. Depression of peripheral NK cell activity could be accompanied by the accumulation of highly active NK-cells in the tumor. The mouse NK cell was cytotoxic for certain human tumor lines. The fit of the mouse NK cell to the human target was not equally good as to the mouse target. In chemically induced mouse sarcoma and rat glioma systems, specific killer T cells could be obtained by diffusion chamber sensitization. Viral "xenogenization" increased the rejectability of chemically induced mouse tumors. In the human system, a fractionation procedure was developed, capable of successfully removing nonspecifically cytotoxic (NSC) cells from the peripheral lymphocyte populations. Fc receptor-positive cells were mainly responsible for nonspecific cytotoxicity. The Fc receptor-depleted T-cell fraction was tested for remaining specific activity in the EBV-IM model system. When isolated from acute mononucleosis patients, it showed a clearly specific cytotoxicity against EBV-carrying, but not against EBV-negative, cell lines.

Significance to Cancer Research: It is essential at this time to distinguish between nonspecific and specific killer effects in both mouse and human tumor systems and to initiate studies on the possible in vivo relevance of in vitro lymphocytotoxicity.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Effects of Streptococcal Serum Factors on Lymphocyte Responses

Principal Investigator: Dr. Janet Plate
Name/Address Massachusetts General Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-53959
Starting Date: 5/1/75 Expiration Date: 9/30/76

Goal: An exogeneous Streptococcus pyogenes C203s product (termed LTF) serves as a mitogen for human peripheral blood lymphocytes. It is our purpose to determine: (1) the specificity of augmenting and inhibitory serum factors and their biochemical nature; (2) association of such serum factors with streptococcal infection; and (3) the site of action and mechanism through which they affect lymphocyte responses.

Approach: Specificity of Serum Effects: One concentration of serum was assayed against a broad range of concentrations of several mitogens. Relationship of Serum Effects and Streptococcal Diseases: Coded sera from 31 individuals with anti-strep antibody activity were studied. Frozen lymphocytes from one donor were cultured in a standard serum and 10% experimental serum. PHA was added. ^{3}H-thymidine incorporation was determined after 86-88 hours of culture. Mechanisms: Identification and purification of cell subpopulation(s) responding to LTF were critical.

Progress: Augmenting serum exhibited its greatest effects on responses to Strep. products including LTF, SK-SD, Grp. A Exotoxin. The serum also increased somewhat responses to LPS and Con A but had no enhancing effect on PHA or PWM responses. The activity is not removed by dialysis or absorptions with human lymphocytes. The suppressive serum examined had a broader effect as Con A, PHA, LTF and SK-SD were reduced by greater than 50%. Depressive effects on PWM and LPS responses were slight. Sera from all acute rheumatic fever patients tested depressed responses to LTF.

Significance to Cancer Research: Cancers and certain other diseases become established despite a functional immune system. In some cases "immunity" is evidenced by specific or nonspecific immunosuppressive serum factors. Knowledge of the mechanisms through which these factors function may further our understanding of "immunity" and tumor cell growth and escape.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Identification, Quantitation and Characterization of Lymphocytes and Macrophages

Principal Investigator: Dr. Nicholas Catsimpoolas
Name/Address: Massachusetts Institute of Technology
Performing Organization: Cambridge, MA

Contract Number: NO1-CB-43928
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: This project focuses on the development of new biophysical techniques for the separation and characterization of cells involved in the immune response, i.e., lymphocytes and macrophages. At the preparative level, these methods should supply sufficient quantities of viable and functional cells for immunological testing. In addition, new analytical instrumentation should provide biophysical parameters useful in the detection of cell subpopulations.

Approach: Physical separation and characterization of cells based on their surface charge and size differences and on the combination of these two properties have been explored. Electrophoresis and velocity sedimentation at 1 g have been utilized as the main experimental tools in achieving separation.

Progress: New methods were developed for the high-resolution separation and analysis of mammalian cells by density gradient electrophoresis and velocity sedimentation at 1 g. Mouse spleen lymphocytes were separated into T and B cells. Surface charge heterogeneity within each of these subpopulations was demonstrated to vary with strain and age of the mouse. Responder and stimulator cells were found only in the high and low electrophoretic mobility fractions, respectively. Human peripheral B lymphocytes bearing IgM and IgD are of low mobility and therefore can be enriched significantly by electrophoresis. Combination of velocity sedimentation and electrophoresis was used to demonstrate the physical heterogeneity of mouse thymus and spleen lymphocyte subpopulations differing in both surface charge and size. Macrophages were separated from lymphocytes in mouse peritoneal exudates by velocity sedimentation at 1 g. Changes in the velocity sedimentation at 1 g of mouse lymphocyte subpopulations upon interaction with specific mitogens at 4°C were measured and proposed as a new tool for the rapid characterization of T-cell subsets.

Significance to Cancer Research: The experimental approaches developed here may lead to the demonstration of the existence of unique subpopulations of lymphocytes in certain forms of human cancer, especially leukemias. They also provide basic cell separation methodology for cancer immunobiology studies in general.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Serologic and Immunogenetic Investigation of Cell Surface Antigens

Principal Investigator: Dr. Ian McKenzie
Name/Address Melbourne University
Performing Organization: Victoria, Australia

Contract Number: NO1-CB-43925
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To define the surface antigens of lymphocytes and tumor cells in the mouse and to determine their genetic and functional interrelationship with the long-term goal of achieving means of selective manipulation of the immune response.

Approach: Inbred strains are immunized to produce antisera which are tested on murine lymphocytes of different types. Antisera are then studied genetically and functionally and reacted with tumors both in vitro and in vivo.

Progress: The original observations of others were confirmed that helper T cells are Ly-1$^+$, 2$^-$, 3$^-$ and killer cells Ly-1$^-$, 2$^+$, 3$^+$. In addition, these studies were expanded into different systems, e.g., viral immunity and T suppressor cell systems (Ts are Ly-1$^-$, 2$^+$, 3$^+$ for antibody formation, but are Ly-1$^+$, 2$^-$, 3$^-$ for delayed hypersensitivity). In addition to Ly-4.2, the Ly-4.1 specificity was described which appears to be present on a subpopulation of B cells and on activated T cells. They are also present on Abelson virus-induced tumors. Ly-5 specificities are being studied in functional assays. They seem to be present on all functional T cells. Ly-6 specificities have been described: They are on T cells, especially on killer T cells. Ly-7 specificities have also been described. These are on B cells and possibly a small subpopulation of T cells. Other specificities such as Tla, Pca, Thy and Ia are also being examined in all of these assays.

Significance to Cancer Research: These studies classify lymphocytes into functional subpopulations and tumors as to site of origin and mode of differentiation. The production of antisera to such tissues provides useful markers and may point the way to a systematic and selective form of immunotherapy.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Dr. Ross S. Basch
Name/Address New York University Medical Center
Performing Organization: New York, NY

Contract Number: NO1-CB-43927
Starting Date: 5/1/74 Expiration Date: 4/30/77

Goal: To isolate and characterize a thymic hormone-responsive thymocyte precursor.

Approach: Testing various populations of hematopoietic cells for their ability to be induced by the thymic polypeptide (thymopoietin). These populations, prepared by a number of physical and serologic techniques, have also been tested for their capacity to repopulate the thymus of irradiated mice.

Progress: The bone marrow and spleen of mice contain cells which would express the differentiation antigens characteristic of thymocytes when treated with thymopoietin, a polypeptide isolated from bovine thymus. These thymopoietin-sensitive cells are indeed thymocyte precursors since they could repopulate the thymus of irradiated mice. These cells are relatively large cells, of low buoyant density, and do not adhere to glass. Unlike their progeny, they are relatively cortisone and γ-irradiation resistant. They lack the surface markers usually associated with either B or T cells. They can be distinguished from other cells in the null cell pool since they have surface antigens that react with antisera against mouse brain. At least two specificities are present, one of which is also present on mature T cells and another which resembles an antigen found on pluripotential stem cells. Attempts are under way to develop immunofluorescent markers for these cells so they may be more readily identified. A method has been devised for the fractionation of bone marrow and spleen cells which has enabled production of samples in which the prothymocyte content is enriched by 40-fold.

Significance to Cancer Research: Thymocyte precursors seem to be the cells "at risk" in the development of most murine and some human leukemias. Immunotherapy for any malignant disease will ultimately require the ability to influence selectively the T-cell compartment. Knowledge about the precursors of this compartment seems obligatory.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Purified Thymic Products Promoting Lymphocyte Differentiation

Principal Investigator: Dr. Gideon Goldstein
Name/Address New York University Medical Center
Performing Organization: New York, NY

Contract Number: NO1-CB-53868
Starting Date: 8/15/74 Expiration Date: 8/14/77

Goal: To determine the chemical structure of thymopoietin, with particular emphasis on the portions responsible for biological activity in inducing thymocyte differentiation.

Approach: The isolation of bovine thymopoietin was scaled up to produce adequate amounts for biochemical characterization. The complete amino acid sequence was determined (primary structure). Attempts to determine the primary and tertiary structure of the active site region by a combination of approaches are under way.

Progress: Synthesis of 13 of the 49 residues of sequence of thymopoietin has produced an active molecule. Further studies are under way to determine if still shorter fragments are active. Small crystals of thymopoietin have been obtained and it is hoped to grow crystals adequate for analysis of tertiary structure by x-ray diffraction techniques. Nuclear magnetic resonance is being employed to further define the solution structure of the molecule.

Significance to Cancer Research: Present evidence indicates that thymopoietin not only induces early thymocyte differentiation but also modifies the reactivity of immunocompetent cells. Active peptides or analogs may, therefore, be of therapeutic value as immunostimulants in cancer patients.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 6/74
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Dr. Gerald Bartlett
Name/Address
Performing Organization: Pennsylvania State University
Milton S. Hershey Medical Center
University Park, PA

Contract Number: NO1-CB-64039
Starting Date: 6/16/76 Expiration Date: 6/15/77

Goal: To test whether "spontaneous" murine tumors appear to be nonantigenic due to processes which are known to interfere with cell-mediated cytotoxicity.

Approach: Use tumors shown to be nonantigenic in previous tests, and obtain new tumors (spontaneous and induced) expected to be nonantigenic. Test the antigenicity of tumors in immunization-challenge experiments designed for maximal sensitivity, and compare those data with results in the Winn test, which may have additional sensitivity. Use the Winn test to assess tumor suppressive properties of lymphoid cells from sensitized animals after various manipulations intended to (1) deplete suppressor cells, (2) remove blocking factors, or (3) "reactivate" sensitized cells.

Progress: Four spontaneous tumors (one rat histiocytic lymphoma, one rat lymphocytic lymphoma, one mouse lymphocytic leukemia and one mouse epidermoid carcinoma) have been obtained from animals in our colony. Approximately 10 primary spontaneous mouse tumors have been obtained from the Jackson Laboratory. Groups of mice have been established to obtain new primary tumors of several classes expected to be weakly antigenic or nonantigenic: Spontaneous and induced pulmonary adenomas, sarcomas induced by smooth films, tumors developing from spontaneous transformation in diffusion chambers, and endogenous hormone-induced tumors. Sensitive antigenicity tests and Winn tests have confirmed the nonantigenicity of two spontaneous diffusion chamber tumors studied previously.

Significance to Cancer Research: Many classes of tumors, especially spontaneous animal tumors, are "nonantigenic" when examined in in vivo tumor rejection tests. When such "nonantigenic" tumors are studied by serologic or by in vitro cell-mediated immunity tests, they frequently express tumor-associated antigens. Apparently the immunity that can be detected in vitro is ineffectual for tumor rejection in vivo. This project is to examine the role of several processes which might be interfering with tumor rejection immunity. Such information would guide subsequent efforts to improve methods for immunotherapy of cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells in Tumor Masses

Principal Investigator: Dr. John W. Kreider
Name/Address Pennsylvania State University
Performing Organization: Milton S. Hershey Medical Center
Hershey, PA

Contract Number: NO1-CB-43931
Starting Date: 5/1/74 Expiration Date: 4/30/77

Goal: To identify the most important features of the leukocytic infiltrates of tumors regressing as a result of a specific immunological response.

Approach: Regressing with progressing tumors was compared in three animal models: (1) autochthonous Moloney sarcomas in which regression occurs in young adults but tumors progress in newborns; (2) line 1 hepatoma in which regression occurs in transplants to skin but tumors progress in intramuscular sites; (3) line 10 hepatoma in which regression occurs only as a result of treatment with a tumor cell-BCG vaccine. Regressing tumors and age-matched progressing controls were dissociated with collagenase and leukocytes identified with appropriate markers.

Progress: Studies in previous years demonstrated that in the Moloney sarcoma, regressing tumors contain significantly more membrane IgG-positive and EA rosette-forming leukocytes than progressing tumors. Morphologically, these cells were large lymphocytes and macrophages. The T-cell population was not increased. In the present contract year, it was found that trypan blue, a putative inhibitor of macrophage cytotoxicity, did not prevent Moloney sarcoma rejection but did block the rejection of tumor allografts differing at multiple minor loci. Surprisingly, the leukocytic infiltrates of regressing and progressing line 1 hepatomas did not differ in the numbers of E or EA rosette-forming cells, membrane IgG or ATS-positive cells, phagocytic cells, or nonspecific esterase-positive cells. Trypan blue did block the line 1 regression, suggesting that macrophage function may be important. The line 10 hepatoma, vaccine-induced regressing tumors contained significantly more T cells than progressing tumors, but numbers of other cell types were not different. Trypan blue also blocked line 10 regression. T cells were identified in the infiltrate which expressed Fc receptors, although it is not clear whether they differ in progressing and regressing tumors.

Significance to Cancer Research: Pathologists have observed that an inflammatory response in human tumors appeared to be associated with a favorable prognosis. An objective, quantitative test of this hypothesis in representative animal tumor models will be provided.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Liposome Model System

Principal Investigator: Dr. Arnold Sanderson
Name/Address Queen Victoria Hospital
Performing Organization: McIndoe Research Unit
East Grinstead, Sussex, England

Contract Number: NO1-CB-53876
Starting Date: 6/16/75 Expiration Date: 6/15/77

Goal: To study the incorporation of products of the major histocompatibility complex (MHC) of rat and man into synthetic liposomes, and to examine the immune mechanisms capable of damaging such liposomes, to provide a model for studying the ways in which cells bearing foreign antigenic markers are eliminated, or avoid elimination, by the host's immune system.

Approach: Purified membrane proteins may be reintegrated into synthetic lipid bilayers provided that the lipophilic portion of the molecule is preserved intact. The contractor solubilized products of the MHC from spleen membranes and inserted these purified proteins into synthetic lipid bilayer.

Progress: The first year's work concentrated on purification of rat and human MHC products. Antigen is now prepared by solubilization of spleen cell membranes with 1% sodium cholate followed by gel filtration and lectin affinity chromatography. When solutions of antigen plus lipid in cholate are rapidly diluted, liposomes containing protein are formed, which may be isolated by centrifugation. All the antigenic activity is available for antibody binding, indicating that the antigen assembles asymmetrically with respect to the bilayer. It has been possible to prepare liposomes bearing MHC antigens and containing trapped horseradish peroxidase or lysozyme. These liposomes may be specifically agglutinated by anti-MHC sera, and preliminary experiments show that they may be lysed by the action of specific antibody plus complement, using the release of enzyme as a marker for membrane impairment. Rat AgB antigens inserted into liposomes have been shown to provoke a powerful secondary response, but to date, it was not possible to stimulate a primary response.

Significance to Cancer Research: The work of Zinkernagel and Doherty and others has suggested that the immune elimination of virus-infected cells, and, by implication, tumor cells, proceeds via recognition of the cell's MHC antigens, either as "altered-self" products, or in some form of "dual recognition." This contractor's model is ideally suited for studying such immune reactions.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Dr. R. L. Marquet
Name/Address Radiobiological Institute
Performing Organization: Rijswijk (KH), The Netherlands

Contract Number: NO1-CB-64038
Starting Date: 6/16/76 Expiration Date: 6/15/77

Goal: Investigations on the immunogenicity of tumors in syngeneic rats. The intent is to survey a large variety of tumors that share the following characteristics: (1) arise spontaneously in low frequency and (2) arise following exposure to various dosages of irradiation.

Approach: The ultimate tests of immunogenicity will be in vivo challenge-protection experiments in syngeneic rats, using early transplant generations of the tumors.

Progress: In the period of June 1976 to January 1977, a total of 80 rat tumors from WAG/Rij, SD and BN rats became available and were transplanted isogeneically. Thirteen tumors were "spontaneous" tumors, 50 tumors were mammary tumors derived from irradiated rats, and 17 were non-mammary tumors also harvested from irradiated rats. Of these 80 tumors, 47 grew to a palpable size in 1-6 months in the first passage. From this group of 47 transplantable tumors, 7 tumors were tested for immunogenicity in challenge-protection experiments. Two were mammary tumors derived from irradiated WAG/Rij rats, 2 were mammary tumors from irradiated BN rats, 1 was a BN-round cell sarcoma of the cervix (irradiated animal), 1 was a WAG/Rij neurofibrosarcoma (irradiated animal), and 1 was a "spontaneous" WAG/Rij adenocarcinoma. From the 7 tumors which have thus far passed the first experimental approach, two tumors, a WAG/Rij fibroadenoma and a BN-fibrosarcoma, are of special interest since the growth curves obtained were suggestive of tumor immunogenicity.

Significance to Cancer Research: Several laboratories have reported that "spontaneous" animal tumors have little or no immunogenicity as measured by challenge-protection experiments. If this observation is generally correct, it would have important implications for ideas about immunotherapy and immunodiagnosis.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. W. Hallowell Churchill
Name/Address Robert B. Brigham Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-64036
Starting Date: 5/30/76 Expiration Date: 6/29/77

Goal: To determine if sensitized lymphocytes stimulated with tumor antigen generate macrophage-activating factor (MAF) or factors capable of enhancing macrophage-mediated cytotoxicity. To further define the nature of the interaction between MAF and macrophages. To explore the mechanisms by which macrophages, once activated, kill tumor cells.

Approach: Macrophages are activated in vitro by treatment in suspension culture with MAF-rich supernatants. Their cytotoxicity for tumor cells labeled with ^{3}H-proline or ^{3}H-thymidine is then determined.

Progress: Studies have shown that enhancement of cytotoxicity can be demonstrated after as little as 8 hr incubation. Fucose and galactose inhibit the effect of MAF on macrophage cytotoxicity if tested after short periods of activation (15 hr), but this effect disappears after a more prolonged incubation. A short-term cytotoxicity assay has been developed so that it is now possible to study the effect of removing surface sugars from macrophage membranes on mediator-induced macrophage cytotoxicity. Intact protein synthesis, glycolysis, microtubular and microfilament function are required for macrophage-mediated cytotoxicity. No evidence was found that macrophage cytotoxicity is mediated by soluble factors in that supernatants from macrophages activated by mediators or endotoxin were as toxic to target cells as control supernatants were. Finally, increased adherence of normal macrophages to tumor cells by formation of Schiff bases is associated with enhanced cytotoxicity of the tumor cells.

Significance to Cancer Research: Macrophages are an important component of the host response to tumor antigens. Our studies are designed to elucidate the mechanisms by which macrophages acquire enhanced cytotoxicity for tumor cells and the mechanisms by which activated macrophages kill tumor cells.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Role of the Macrophage in Tumor Resistance

Principal Investigator: Dr. D. S. Nelson
Name/Address Royal North Shore Hospital
Performing Organization: Kolling Institute of Medical Research
St. Leonards N.S.W., Australia

Contract Number: NO1-CB-63973
Starting Date: 7/16/75 Expiration Date: 10/31/77

Goal: The aims of this work are to determine what roles macrophages may play in resistance to tumors and whether they can be exploited in immunotherapeutic strategies.

Approach: The growth of grafts of chemically induced tumors is measured in the feet of mice. Studies are made of the effects of various agents and procedures on the growth rate of tumors in normal and immune mice. The mice are immune either (a) during carriage of a tumor (concomitant immunity, at first specific and later nonspecific) or (b) after injection of a small number of tumor cells (sinecomitant immunity, always specific). In parallel, the effects of macrophages and other cells on the survival and multiplication of cultured tumor cells are measured.

Progress: Agents depressing macrophage activity (e.g., silica) in intact animals depressed specific concomitant or sinecomitant immunity, but not nonspecific concomitant immunity. Specific immunity was also depressed by agents depressing delayed-type hypersensitivity (e.g., carrageenan, niridazole, reserpine). Nonimmunized mice offered some resistance to the growth of tumor grafts; this was depressed by some agents depressing macrophage function. All mouse, rat, and human tumor cells tested were found to produce a factor(s) antagonizing macrophage activity *in vivo* and *in vitro*. Excessive amounts of this factor(s) accelerated tumor growth in nonimmune mice. Resistance to the factor was associated with resistance to tumor growth. Tumor immune mice do not possess cells capable of causing rapid death of tumor cells. Two types of cells have been found to attack cultured tumor cells: one, perhaps a small macrophage, causes slow death of tumor cells; a large "activated" macrophage slows or stops the growth of tumor cells. Both types suppress tumor growth in intact animals.

Significance to Cancer Research: Broadly, these results suggest that (a) macrophages are important in resistance to tumors; (b) immune attack on tumor cells is relatively inefficient; (c) an antagonistic effect on macrophages is a common property of tumor cells; and (d) overcoming this effect promotes resistance to tumors.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $76,456

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune-Related Cell Lines

Principal Investigator: Dr. Melvin Cohn
Name/Address
Performing Organization: The Salk Institute
San Diego, CA

Contract Number: NO1-CB-23886
Starting Date: 2/26/74 Expiration Date: 2/25/78

Goal: To supply the scientific community with immune-related lines important in the study of tumor immunology and to increase the library of useful lines as they are characterized in our laboratory.

Approach: Since this is essentially a contract to supply cell lines, there is little experimental work involved. However, the general thrust of the laboratory involved the isolation of immune-related lines of the thymus and bone marrow-derived lineages by the use of leukemia viruses, cell fusions, and chemical carcinogens. As new lines become available, they are introduced into the contract.

Progress: Immune-related lines are usually dead-end and do not grow in culture. It is necessary therefore to transform them in order to isolate and clone them in vitro. The contractor has a vast experience with the induction of myelomas, lymphomas, macrophage tumors, and other immune-related cells using chemical carcinogens and leukemia viruses. This work has formed the basis for the present contract to supply cell lines. During the present contract year, these studies were extended to isolation of normal cells using cell fusion techniques. Antibody-producing cells as well as T cells of the cytotoxic and cooperating lineages were isolated.

Significance to Cancer Research: The lines in this contract are used by hundreds of laboratories for studies on tumor-specific antigens, antibody structure, immune-related cell functions, somatic genetics, and cell fusions. All of these subjects are major categories under the NCI Cancer Research Program and the cell lines from this laboratory are key tools in these investigations.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $68,600

CONTRACT RESEARCH SUMMARY

Title: Supramolecular Organization of Normal and Tumor Cell Surfaces

Principal Investigator: Dr. Garth L. Nicolson
Name/Address The Salk Institute
Performing Organization: San Diego, CA

Contract Number: NO1-CB-33879
Starting Date: 6/25/73 Expiration Date: 9/24/77

Goal: To study the cell surfaces of normal, transformed, and tumor cells of differing malignant or metastatic potential. To determine what surface changes occur and how these changes relate to the detection of tumor cells by a host immune system, *in vivo* killing of tumor cells, escape from growth regulation, and metastatic spread.

Approach: Use and characterization of new lectins for labeling and purification of surface glycoproteins. Use of toxins that show selective killing of transformed cells. Characterization and isolation of neuraminidase-sensitive surface receptors from tumor cells.

Progress: One lectin (peanut agglutinin) appears to specifically react with mammary carcinoma and should be useful for the purification of mouse, rat, and human carcinoma glycoproteins. An 80,000 mol. wt. glycoprotein was identified that is responsible for the transport of RCA_{II} toxin to sensitive mouse and human lymphoma cells. Resistant variants have been isolated which are either lacking or have a modified 80,000K component. Cell surface components bearing neuraminidase-sensitive sialic acid have been isolated and purified using peanut lectin affinity chromatography with murine MT12 mammary adenocarcinoma. High metastatic melanoma and mammary carcinoma release greater amounts of cell surface-associated glycoproteins *in vitro* compared to variants of low *in vivo* metastatic potential. Organ selectivity appears to take place through protease-sensitive surface components which are probably glycoproteins used for blood-borne endothelial cell implantation.

Significance to Cancer Research: Understanding specific tumor cell surface receptors involved in these processes is of primary importance in understanding tumor progression. Specific cell surface receptors appear to be involved in metastatic tumor spread. Understanding the structure and dynamics of these receptors may aid in the prevention of metastatic cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $146,212 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells in Tumor Masses

Principal Investigator: Dr. Stephen W. Russell
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: NO1-CB-44001
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To characterize the inflammatory cells found in solid murine tumors.

Approach: Methods were developed to disaggregate tumors enzymatically and to quantify the inflammatory cell types in resultant cell suspensions. Other techniques then were developed to isolate the principal mononuclear inflammatory cell types found within tumors, and to test them in vitro for their functional activities.

Progress: The contractor developed and applied the methodology needed to obtain representative populations of constituent inflammatory cells from tumors. The best disaggregation techniques were shown to be enzymatic. Methods of identification which were applicable to enzyme-treated cells, as well as techniques which allowed the recovery of purified populations of functionally intact inflammatory cells had to be developed. The functions of cells recovered directly from regressing or progressing Moloney sarcomas were tested. T lymphocytes found in the neoplasms are capable of killing in an antigenically specific manner, while the macrophage is capable of killing in an antigenically nonspecific manner. Both T lymphocytes and macrophages recovered from progressing sarcomas are less capable of killing than are T cells and macrophages from spontaneously regressing neoplasms of the same type.

Significance to Cancer Research: These studies are of significance for three reasons: (1) They have provided the methodology needed to disaggregate tumors, and to quantify and recover the inflammatory cell types contained therein; (2) they have called attention to functional differences which may exist between the same inflammatory cell types contained in regressing vs. progressing neoplasms; and (3) they have emphasized the fact that successful rejection of neoplastic cells in vivo is undoubtedly dependent on a complex interplay between a number of inflammatory elements, rather than any one cell or antibody type.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $5,571

CONTRACT RESEARCH SUMMARY

Title: Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator: Dr. Stuart Schlossman
Name/Address Sidney Farber Cancer Center
Performing Organization: Boston, MA

Contract Number: NO1-CB-53881
Starting Date: 5/1/75 Expiration Date: 4/30/77

Goal: Develop or improve techniques for in vitro sensitization of human lymphocytes against syngeneic or autologous tumor-associated antigens in order to increase capability for studying cellular interactions involved in the production of cytotoxic cells and ultimately aid in designing new therapeutic approaches to cancer.

Approach: Investigation of sensitization of human lymphocytes to syngeneic and autologous leukemic and solid tumor cells using lymphocytes from both patients and HLA-MLC matched siblings.

Progress: The contractor has identified and isolated the subclass of human T cells important in the response to alloantigens. They demonstrated that cytotoxic cells specifically recognize the serologically defined antigens controlled within the major histocompatibility complex (MHC). Normal cytotoxic lymphocyte populations do not induce significant cytotoxicity on MHC identical normal lymphocytes or PHA blasts even in the presence of an unrelated third-party LD stimulant. Among other studies were experiments done to evaluate the destruction of leukemic blasts by MHC identical siblings. Where acute leukemic cells were unable to induce a significant proliferative response by MHC identical lymphocytes, the generation of effective, specific anti-leukemic cytotoxicity required the addition of unrelated stimulating cells to the sensitization cultures. On the other hand, leukemic blasts that induced a proliferative response by MHC identical lymphocytes were able to stimulate specific anti-leukemic cytotoxicity (which could be augmented by the addition of unrelated stimulating cells to the sensitization cultures).

Significance to Cancer Research: NCP Objective 6 Approach 4.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $64,648

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Fung-Win Shen
Name/Address Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, NY

Contract Number: NO1-CB-64031
Starting Date: 6/16/76 Expiration Date: 6/15/77

Goal: To develop a system for measuring the immune capability of T cells against syngeneic chemically induced tumors of C57BL/6 mice in vivo. By use of this system to delineate the contribution of tumor-specific T cells to immunity against tumors in vivo. To determine which functionally distinct T-cell populations may be responsible for abrogating this destruction in vivo. To determine whether any cross-reactions of different chemically induced tumors can be demonstrated by cellular as well as serological methods.

Approach: The in vivo assay will be applied to a series of chemically induced C57BL/6 transplanted tumors to establish the role of the Lyt-1, Lyt-23 and Lyt-123 functional subclasses, alone and in combination, in the immune response to chemically induced tumors in vivo.

Progress: It has been established that it is the nonadherent rather than the adherent cells which are responsible for suppression in the Winn-type assay; it is the theta-positive cells within this population that are responsible for rejection; and it has further been shown that elimination with either anti-Lyt-1 or anti-Lyt-2 also abolishes the efficacy of the nonadherent Thy-1^+ cells.

Significance to Cancer Research: The ultimate control of cancer by immunological means may depend on an intimate knowledge of how the different functionally distinct T-cell subpopulations may conspire to generate effective tumor-destructive immune response in vivo. A foreseeable outcome of this study is that elimination of one functionally distinct Lyt cell population may augment tumor rejection, and another suppress it. At this point it would not be difficult to envisage research programs directed to the application of such methods to therapy of at least some human cancers.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens and Corresponding Antibodies

Principal Investigator: Dr. Yashar Hirshaut
Name/Address Sloan-Kettering Institute for Cancer
Performing Organization: Research
New York, NY

Contract Number: NO1-CB-53942
Starting Date: 5/30/75 Expiration Date: 6/29/77

Goal: To demonstrate new tumor-related antigens associated with human sarcomas. Antigens found, as well as those already demonstrated, are to be characterized to determine their nature, specificity, and distribution.

Approach: The serologic responses of patients with sarcoma to their tumor cells are assayed by immunofluorescence, complement fixation, and microcytotoxicity tests. Preliminary characterization of antigens is achieved by immunologic means. Efforts are under way to isolate, purify, and biochemically analyze specific antigens.

Progress: To provide sufficient substrate for this study, 72 cell lines have been established. Many of these lines cytologically resemble the tumor of origin. In addition to the previously identified antigens, S_1, S_2, and S_3, serologic reactivity has been noted by means of microcytotoxicity. S_1 and S_3 have been shown to represent new heterophile substances. The S antigens have been distinguished from known heterophile and tumor antigens including CEA and alpha-fetoprotein. S_3 has been prepared in sufficient quantities to permit purification and biochemical analysis to proceed. The nature and specificity of this antigen are as yet unknown.

Significance to Cancer Research: Definition and characterization of human tumor antigens should be of value in permitting early diagnosis and the further development of immunotherapy for cancer. Such antigens may also provide new insight into changes that accompany malignant transformation.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Automated Systems for HLA Typing

Principal Investigator: Dr. F. Carl Grumet
Name/Address Stanford University
Performing Organization: Stanford, CA

Contract Number: NO1-CB-53935
Starting Date: 5/16/75 Expiration Date: 9/29/77

Goal: To complete the final phase of development of an automated HLA typing system and to demonstrate the effectiveness of this system.

Approach: Trays are washed and typed. Total fluorescence in each well is proportional to total cell viability, so that test reading is converted from an enumerative process to a machine-readable quantitative process.

Progress: The automated system under study was based upon a modification of a standard lymphocyte microcytotoxicity assay using a fluorescent marker of cell viability. Data analysis programs using relatively inexpensive minicomputers to handle data obtained with the automated typing system. The hardware and software for a stepping motor microscope stage and interfaces are working, with 72 reactions read directly into the computer from the test plate in 100 seconds. Programs to handle data have reduced analysis time by 25%, and a quantitative micromethod for titration of antisera was developed. Concordance between visual and automated readings remains 95%. The automated scope has also been applied to testing sera for B-lymphocyte alloantigens. A multiple carrier for processing typing plates is under construction. Software programs have reduced analysis time by 80% and also expanded minicomputer capability so that both pattern recognition and Chi square programs are run on the PDP8. A preliminary experiment has been shown that mixed lymphocyte culture reactions (HLA-D locus typing) may be quantitated by the automated scope. Preliminary data shows that B-cell typing and possibly D-locus typing may be performed with the automated scope.

Significance to Cancer Research: HLA typing is important in research focusing on genetic determinants of disease susceptibility, particularly since animal models clearly demonstrate that genes linked to the species MHS can affect susceptibility to different viral-induced tumors. An improved ability to perform HLA typing should permit fine genetic mapping of the MHS and possibly help localize and identify any human disease susceptibility or immune response genes in that MHS region.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $42,234 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Recombinant Inbred Strains of Mice for Analysis of Ir-1, Rgv-1 and Linked Genes

Principal Investigator: Dr. Hugh O. McDevitt
Name/Address Stanford University
Performing Organization: Stanford, CA

Contract Number: NO1-CB-43941
Starting Date: 3/1/74 Expiration Date: 2/28/77

Goal: To develop and test mice bearing recombinant H-2 alleles in relation to analysis of the immune response.

Approach: The contractor will develop and maintain large breeding colonies of inbred mice for use in production and testing of antisera directed against a variety of known H-2 recombinants.

Progress: In addition to being able to meet requirements for production of mice and a large number of anti-Ia sera combinations, the contractor met increasing demands from other research laboratories for supplies of animals and antisera. In addition, a number of virus-induced thymomas have been obtained and adapted to tissue culture. These thymomas have been developed to permit a search for specific immune response genes recognizing virus or tumor transplantation antigens. The availability of such cells permits analysis of possible associations between viral antigens and H-2 determinants in a variety of distinct haplotypes. During the past year definition of immune response or immune suppressor gene(s) affecting cell-mediated lysis of virus infected cells has been tentatively mapped to the I-J subregion of the complex. In addition, the mechanism of D-region-associated genes in conferring resistance to virus-induced leukemia has begun to be elucidated.

Significance for Cancer Research: (NCP Objective 2 Approach 3) Studies of genetically influenced immune responses are an important part of the Baseline Information Flow of the Tumor Immunology Contract Program.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Nature of Interactions Between Tumor Cells and Immunoglobulins

Principal Investigator: Dr. Isaac P. Witz
Name/Address Tel Aviv University
Performing Organization: Tel Aviv, Israel

Contract Number: NO1-CB-43858
Starting Date: 8/15/73 Expiration Date: 8/14/76

Goal: To evaluate expressions of humoral anti-tumor immunity within the tumor site and to understand how the microenvironment of the tumor influences such expressions.

Approach: Immunochemical and serological characterization of immunoglobulins bound to, and Ig-binding cells within, murine tumors. Influences of tumor-derived proteases on the physicochemical characteristics of anti-tumor antibodies and on their capacity to mediate complement-dependent cytotoxicity.

Progress: Certain in vivo propagating murine tumor cells are coated in vivo with potentially cytotoxic antibodies. Anti-tumor antibodies eluted from these murine tumors express several (sometimes conflicting) biological activities such as mediation of complement-dependent cytotoxicity, synergism with lymphocytes in cell-mediated cytotoxicity or blocking of lymphocyte stimulation by tumor cells. Murine tumors contain Fc-receptor positive cells. These are host-derived immunocytes but certain nonlymphoid tumor cells were also shown to express Fc receptors. Tumor-derived proteases degrade immunoglobulins. Degradation products of anti-tumor antibodies which lost the capacity to mediate complement-dependent cytotoxicity block such a cytotoxicity mediated by undergraded antibodies.

Significance to Cancer Research: Thorough evaluation of the immunological relationship between the tumor bearer and its neoplasm is an essential prerequisite for a rational approach to cancer therapy and to a correct assessment of prognosis. In situ anti-tumor immunity and the influences of the tumor's microenvironment on this immunity are indispensable parameters of such an evaluation.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. Yves Borel
Name/Address Tufts University
Performing Organization: New England Medical Center
Boston, MA

Contract Number: NO1-CB-43970
Starting Date: 5/1/74 Expiration Date: 4/30/77

Goal: To study the cellular mechanism of tolerance to determine whether the principle of carrier-induced tolerance can be applied to a tumor-specific transplantation antigen (TSTA).

Approach: Consists of linking a nonimmunogenic determinant such as the hapten (DNP) to isologous mouse gamma globulin (i.e., the tolerogen) to induce tolerance in adult animals to the same hapten linked to a heterologous carrier protein (i.e., the immunogen). Since the tolerogen remains on the surface of the antigen-binding cell in vivo, while the immunogen does not, the cellular mechanism of tolerance can be studied specifically. Furthermore, it was determined whether BALB/c mice treated with TSTA linked to isologous IgG would facilitate the growth of a tumor induced by SV40 and bearing TSTA on its surface.

Progress: It was found that the tolerogen binds with greater avidity than the antigen to the receptor of the hapten-binding cell. Both the carrier and the position of the hapten on the carrier are critical factors in tolerance induction. Carrier-determined tolerance is reversible in vitro. The cellular mechanism involves both T and B cells. The presence of tolerant cells is directly related to tolerance in vivo. Thus, when tolerance wanes, receptor blockade is lost. Anti-carrier antibody breaks tolerance to the hapten in vivo. Thus, tolerance to an antigenic determinant may be broken by an antibody directed not to this determinant, but to another on the same molecule. TSTA was covalently bound to isologous BALB/c IgG. The influence of TSTA-IgG treatment in BALB/c mice on the development of cellular immunity elicited by SV40 and/or tumor growth of TSTA tumor-bearing cell was determined. It was found that TSTA-IgG failed to influence either cellular immunity elicited by SV40 or tumor growth of TSTA tumor-bearing cells.

Significance to Cancer Research: This contract had provided new information on the cellular basis of tolerance using both functional and morphologic techniques, which might be directly relevant to the immunologic surveillance theory.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Dr. Donald F. H. Wallach
Name/Address Tufts University
Performing Organization: New England Medical Center
Boston, MA

Contract Number: NO1-CB-44000
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: Fractionation and characterization of plasma membranes of normal and SV40 transformed lymphocytes and study of their membrane dynamics.

Approach: Cell characterization morphologically and karyotypically, membrane characterization of biophysical spectroscopy. Cell disruption by nitrogen decompression; membrane purification by differential, polymer-density gradient and affinity-density perturbation centrifugation. Membrane protein analysis by SDS-PAGE, isoelectric focusing, crossed immune electrophoresis, bidimensional focusing-SDS-PAGE, bidimensional focusing-immune electrophoresis.

Progress: The following was achieved under the contract: Characterization of karyotypes of normal and transformed lymphocytes and their surface topology. High yield purification of equivalent plasma membrane vesicles from normal and transformed cells. Characterization of plasma membrane proteins from normal and SV40-converted cells; documentation of qualitative differences by SDS-PAGE, isoelectric focusing and crossed immune electrophoresis; demonstration of increased side chain amidation and glycoprotein sialylation in transformed-cell membrane proteins. Identification of new components in plasma membranes of SV40-converted cells as glycoproteins of isoelectric points (pI) 4.7 and 4.5, respectively, with molecular weights of ~100,000 and 58,000, respectively. Characterization of the pI 4.7 and pI 4.5 components as SV40-specific U- and S/transplantation/antigens. Spectroscopic (Raman) demonstration of profound differences between two membrane categories in terms of thermotropic lipid- and protein-state transitions. Molecular characterization of SV40-membrane antigens and correlation with anomalous membrane protein and lipid dynamics. Indication that virus gene products distribute, after post-translational modification, into all membrane components of transformed cell.

Significance to Cancer Research: Molecular clues to pleiomorphic membrane alterations in cancer, correlated to tumor immunology.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Dr. Paavo Toivanen
Name/Address Turku University Medical School
Performing Organization: Turku, Finland

Contract Number: NO1-CB-43971
Starting Date: 6/1/74 Expiration Date: 6/30/77

Goal: To determine origin and differentiation mechanisms of prebursal and bursal stem cells in the chicken, and to elucidate mechanisms of T-cell differentiation during early ontogeny in man and sheep.

Approach: In the studies on B-cell differentiation, the main approach has been transplantation of cells into immunodeficient chicks or chicken embryos, and evaluation of the effects of transplantation by functional and morphological means. Studies on T-cell differentiation are based on in vitro cultures of embryonic cells.

Progress: It was shown that only embryonic cells capable of reconstituting 18-day cyclophosphamide-treated chicken embryos are bursa cells. No evidence for the yolk sac origin of prebursal stem cells has been obtained. Development of germinal centers in the chicken has been proved to be dependent on the cooperation of histocompatible T and B cells.

Specifically recognizing T lymphocytes were shown to appear in the embryonic human and sheep thymus within 1-2 weeks after the thymus became lymphoid. Strong allogeneic responses by prethymic liver cells were shown to be immunologically nonspecific. Specific effector cells for cell-mediated lympholysis were demonstrated to appear during the intrauterine life of human fetus.

Significance to Cancer Research: To understand disturbances in the immune surveillance system facilitating the development of malignancies, and to be able to manipulate the system therapeutically, detailed knowledge of its normal development is of fundamental importance.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $59,000 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. J. Claude Bennett
Name/Address University of Alabama
Performing Organization: Birmingham, AL

Contract Number: NO1-CB-44009
Starting Date: 6/1/74 Expiration Date: 11/30/76

Goal: Develop methodologies and approaches which are expected to have wide application in studies of macromolecules present at cell surfaces. Correlate these studies from the standpoint of both structure and function. The model system used was the TL antigen system of the mouse. Work undertaken included antigen preparation and purification and relevant biochemical studies.

Approach: Development of techniques for papain solubilization of the TL antigen, the definition of its subunit structure, the definition of sites and mechanism of cleavage from the cell surface and general work toward a definition of a model for the structure of the molecule.

Progress: During the contract period, significant progress was made in several areas by the investigators. These included: (1) Final assembly of our mass Cell Culture Center and the development of capabilities to produce 10^{12} cells per week and the adaptation of several murine lymphoblastoid cell lines for growth within this setting. Although this Center was established by a grant from the National Science Foundation, large numbers of cells for adequate quantitative biochemistry were absolutely essential to fulfill the commitments of this contract. (2) Development of more accurate assay methods for evaluation of cell surface antigens in the presence of detergent (glutaraldehyde-aggregated cell surface antigens followed by radioimmune absorption). (3) Development of sensitive biochemical techniques for the routine sequencing of peptides at the sub-nanomole level.

Significance to Cancer Research: Develop new, sensitive microchemical methods suitable for use in characterization of cell surface antigens. This has general application in a wide range of areas including genetically determined antigens, tumor antigens, and embryonic differentiation antigens.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Analysis of Serum Requirements for In Vitro Immunological Studies

Principal Investigator: Dr. Robert I. Mishell
Name/Address University of California
Performing Organization: Berkeley, CA

Contract Number: NO1-CB-23883
Starting Date: 6/28/72 Expiration Date: 8/31/77

Goal: (1) To screen commercial preparations of fetal calf serum (FCS) to identify those which are suitable for in vitro cellular immunological studies, and (2) to develop methods for increasing the supply of satisfactory sera.

Approach: (1) Available commercial lots of FCS are tested for capacity to support primary immune reactions in tissue culture and information on suitable lots is made available to all concerned scientists. (2) A bacterial contaminant found in satisfactory sera is being investigated as a probable source of an adjuvant to convert deficient FCS into FCS which is suitable for supporting in vitro immune responses.

Progress: Background: Only 5-10% of commercially produced lots of FCS are suitable for the generation of immune responses in tissue culture. The unsuitable lots are deficient, yet nontoxic. This contract supports work to identify suitable lots for the scientific community and to investigate methods for converting deficient into satisfactory sera. Summary of Results: Several hundred lots of sera have been screened and information about satisfactory lots has been made available to more than one hundred laboratories throughout the world. A cold growing bacterium discovered as a contaminant of a particularly good lot of serum was found to have the capacity to convert deficient into supportive FCS. The organism was identified as a gliding bacterium, probably of the Cytophaga group. An active cell-free product from this bacterium has adjuvant properties distinct from bacterial lipopolysaccharide and, by itself, the cell-free extract can convert deficient into supportive FCS. It is now being purified for use as a serum supplement and as a specific probe of immune regulation.

Significance to Cancer Research: Much research in immune regulation and its relationship to cancer is done in vitro and depends on the availability of suitable lots of FCS. This contract has led to the discovery of a bacterial product which will facilitate the development of more and better defined FCS reagents for such studies.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 4/25/72
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $72,370 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator: Dr. Eli E. Sercarz
Name/Address University of California
Performing Organization: Los Angeles, CA

Contract Number: NO1-CB-43972
Starting Date: 6/1/74 Expiration Date: 10/31/77

Goal: To understand the role of the various genes involved in the regulation of the T- and B-cell responses to a well-characterized group of protein antigens, the lysozymes; and, hopefully, to understand the submolecular basis for discrimination between non-immunogenic and immunogenic lysozymes.

Approach: Using chicken egg white lysozyme (HEL), its congeners from other gallinaceous egg white, and peptide derivatives, we are studying recognition at the T-cell and B-cell levels. T-cell reactivity has been judged by induction of T-cell proliferation, T-cell rosetting and functional effects -- studies on specificity of suppression and help. B-cell responses are studied by isoelectric-focusing and plaque-forming cell analysis.

Progress: Unresponsiveness to either HEL or HUL results from the presence of two recessive genes within the H-2 locus. One gene, in the K-IA region, may be common for both lysozymes. A second gene, between I-B and D, seems to map differently for the two lysozymes, indicating that at least two different I-J subareas exist. Genetic loci outside of H-2 provide dominant suppressive control over the response level to HEL, HUL and REL. It was found that the non-H-2 effect is controlled by a single genetic locus. We have attributed the lack of response to HEL in $H\text{-}2^b$ mice to a suppressive determinant present on HEL and absent on immunogenic lysozymes. Some helper cells can be raised against HEL, even in $H\text{-}2^b$ mice, and probably are directed against determinants very close to the presumptive suppressor determinant, and are resistant to suppressive cell control. HEL from which the N-terminal and C-terminal have been removed can now prime T cells very effectively in the nonresponding $H\text{-}2^b$ strain.

Significance to Cancer Research: If a strongly suppressive determinant is characteristic of a particular tumor cell, that tumor may be able to gain an irreversible advantage in the battle to overcome host defense mechanisms. Chemical alteration of certain determinants on such a tumor may modulate the suppression.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $90,881

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. Donald A. Rowley
Name/Address University of Chicago
Performing Organization: Chicago, IL

Contract Number: NO1-CB-43998
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To suppress or enhance immunity specifically using complementary idiotypes.

Approach: Complementary idiotypic antibody or cells were tested for their effects on antibody responses, cytotoxicity, and growth of a plasmacytoma.

Progress: In the mouse, autogenously produced or passively given anti-idiotypic antibody profoundly suppresses a primary complementary antibody response but not a secondary or existing response. In the rat, antibody directed against the receptor for alloantigen-suppressed antibody-mediated cytotoxicity in vitro and GVH reactions in vivo. A transplantable plasmacytoma (TEPC-15) induces a transient anti-idiotypic response. Mice immunized with mitomycin-treated plasmacytoma cells produce anti-idiotypic antibody; mice so immunized survive longer. Furthermore, protection afforded was transferred by anti-idiotypic antibody alone. Antibody directed against the TEPC-15 idiotype suppresses cloning and growth of a clonable plasmacytoma having the TEPC-15 idiotype (S107) with either complement alone or with normal effector cells in the absence of complement. The effectiveness of anti-idiotypic antibody is a function of both the density of the idiotype on the target cell surface and the rate of secretion of Ig by the target cell. Anti-idiotypic T cells were induced which inhibit the normal B-cell idiotype response and inhibit cloning and growth of syngeneic myeloma cells bearing the idiotype.

Significance to Cancer Research: Anti-idiotypic regulation provides a mechanism for specific inhibition of a cancer. However, effectiveness will be moderated by the density of the target molecule on target cells and release or secretion of the target molecules. The clonable myeloma provides a well-defined model to study these problems. Work with this well-defined system will help establish general tactics and conditions for obtaining specific immunity against cancers which are not so amenable to analysis.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Studies by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Dr. Eugene M. Lance
Name/Address University of Hawaii
Performing Organization: Honolulu, HI

Contract Number: NO1-CB-63978
Starting Date: 8/16/75 Expiration Date: 8/15/77

Goal: This project investigates the phenomenon wherein animals bearing tumors lose the ability to recruit lymphocytes in response to an immunogenic challenge and, as a consequence, develop a generalized and nonspecific depression of their immunological capacity.

Approach: Cell-free fluids are obtained from tumor-bearing animals and inoculated into tumor-free recipients. These recipients are studied in assays which measure independently the ability to recruit or trap lymphocytes. The immunological performance of such recipients in response to conventional antigens, skin allografts, and tumor challenge is measured as well.

Progress: The transfer of cell-free fluid from tumor-bearing animals (ATF) has been found to interfere with lymphocyte recruitment in otherwise normal recipients. This diminution of trapping is nonspecific in the sense that the effect is independent of the challenging immunogen. ATF-treated animals have a reduction in immune responsiveness, both humoral and cellular. The antibody titers to conventional antigens are reduced and more easily in such animals. Recent studies raise the possibility that some of these effects may be due to a passenger virus present within the tumor lines studied, and this possibility is being intensely studied at the moment.

Significance to Cancer Research: The failure of lymphocyte recruitment and the ensuing generalized immunodepression seen in tumor-bearing animals could interfere with the host's attempts at tumor rejection and also render such hosts less able to ward off infections -- a common clinical concomitant of tumor infestation.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $75,467 (TQ)

CONTRACT RESEARCH SUMMARY

Title: T-Memory Cell Properties Primed to Histoincompatible and Syngeneic Tumor Cells

Principal Investigator: Dr. Pekka J. Hayry
Name/Address University of Helsinki
Performing Organization: Helsinki, Finland

Contract Number: NO1-CB-63977
Starting Date: 7/16/75 Expiration Date: 7/15/77

Goal: To investigate the restrictions of specificity of the secondary T lymphocytes to various allogeneic histoincompatible cells; homing, life-time and recirculating capacity on the secondary T lymphocytes; and mechanism for nonresponsiveness of secondary T lymphocytes to T mitogens.

Approach: Alloantigen-primed secondary T lymphocytes were obtained (1) by size fractionation of MLC blast cells and by reversion of the blasts to secondary lymphocytes on a syngeneic feeder layer; (2) in 1°-MLC culture prolonged over 15 days in conditions where the nonresponding cells do not survive; and (3) by injecting MLC blast cells i.v. to syngeneic recipients.

Progress: In vitro generation of specific memory was obtained. It was also possible to create memory against non-H-2 to antigens. A selective and specific memory response could be demonstrated against the MLC locus product provided that this was presented on stimulator cells carrying partial or total H-2 homology with the responding cells. The homing and life-time of secondary lymphocytes were investigated by injecting the MLC blast cells to syngeneic recipients. Most of the blast cells homed in the spleen and could be recovered from the spleen even 20 weeks thereafter. Shortly (3 weeks) after the transfer, the transferred cells were cytotoxic to relevant target cells in vitro, and elicited a prompt secondary MLC and CML response upon restimulation with the original stimulator cells. Twenty weeks after the transfer, the primed cells slowly returned "back" to resting state, and the secondary response returned to the slow primary pattern.

The mechanism of nonresponsiveness of the secondary T lymphocytes to the T mitogens was studied by separating MLC, PHA, Con A or PWM blast cells at the peak of respective responses and by restimulation of the remaining lymphocytes with the same mitogens or in the MLC. Lymphocytes remaining in culture after removal of PHA or Con A blasts were capable of responding in the MLC and vice versa but not against the primary stimulus.

Significance to Cancer Research: These investigations broaden the overall knowledge of the mechanics of cellular immune response.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Pekka J. Hayry
Name/Address University of Helsinki
Performing Organization: Helsinki, Finland

Contract Number: NO1-CB-64032
Starting Date: 6/28/76 Expiration Date: 6/27/77

Goal: To analyze the origin, type and receptor specificity of allograft-infiltrating cells as well as their functions in the rejection process and later perform similar studies with syngeneic tumor grafts.

Approach: A sponge matrix allograft method was developed enabling complete recovery of the allograft-infiltrating cells in a functionally viable state. A viscous cellulose sponge was infiltrated with strain A fibroblasts and transplanted subcutaneously to the neck of B strain recipient mice. Upon transplantation the sponge behaved as an allograft, and created a vigorous anti-allograft response. The infiltrating cells could be removed from the sponge simply by compression.

Progress: The peak cytotoxicity of the sponge infiltrating cells took place on days 7 to 8 in primary response and on days 6 to 7 in secondary response. A strong lytic activity was observed in draining node and in the spleen in primary response. Allografts are infiltrated by blast cells, lymphocytes, monocytes and macrophages. In syngeneic grafts the inflammatory response was weak. The allograft-infiltrating killer cells were fractionated preparatively. The predominant killer cell in draining node and the spleen was a T lymphocyte (blast), whereas the allograft-infiltrating cell population consisted of several classes of killer cells including T and B lymphocytes, monocytes, and macrophages. The target lysis displayed by the allograft-infiltrating cells was equally specific as that displayed by draining lymph node and spleen cells. This was also experimentally verified in the rat system by using anti-idiotypic antisera and immunofluorescence methods.

Significance to Cancer Research: The findings demonstrate a profound dissociation between systemic and local immunity in allograft rejection.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T Cells

Principal Investigator: V. E. Olavi-Makela
Name/Address University of Helsinki
Performing Organization: Helsinki, Finland

Contract Number: NO1-CB-43923
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To find out whether the receptor antibodies that mediate recognition by T lymphocytes are controlled by the Mendelian genes that control specificity of circulating antibody molecules (V_H genes).

Approach: To determine hapten-specificity of T-lymphocyte reactions of mice that carry an exotic V_H gene. Their circulating antibodies thus have an exotic specificity.

Progress: Before the contract period the contractor had shown that some mouse strains have a genetic peculiarity because of which they produce odd (heteroclitic) antibody to a chemical compound nitrohydroxy phenylacetyl (NP). Genetic evidence suggested that this behavior was controlled by a gene that controls the variable polypeptides of anti-NP antibodies. Prior to the present contract year the contractor found another Mendelian V_H gene that controls a heteroclitic antibody specificity, the V_HABA-HOP^b gene. An anti-idiotype serum against the exotic anti-NP antibody was raised which enabled identification of the T-lymphocyte product in a new way independent of the fine-specificity. The V_HABA-HOP^b gene expresses itself in helper T lymphocytes of the mice that carry it. In collaboration with the group of Rajewsky in Cologne, it was found that T-lymphocyte receptors isolated with the aid of nylon fibers have the fine-specificity and idiotype that circulating antibodies carry. This strongly suggests that recognizing molecules of T-cell antibodies use the same V_H genes as conventional antibodies.

Significance to Cancer Research: Ir genes clearly play an important role in the defense against cancer. This work suggests that Ir genes do not code for the recognition structures of T lymphocytes but have a different function.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $28,800

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. J. H. Schwab
Name/Address University of North Carolina
Performing Organization: Chapel Hill, NC

Contract Number: NO1-CB-43997
Starting Date: 5/16/74 Expiration Date: 11/15/76

Goal: To define bacterial products which can modulate the immune response, to determine their mechanism of action, and to measure their influence on tumor development in mice.

Approach: An immunosuppressant factor is isolated from protoplast membranes of group A streptococci and the immunopotentiating peptidoglycan is isolated from the cell walls of these bacteria. The effect of these materials on B cells, T cells and macrophages is measured in vitro. The modification of tumor growth in vivo is measured by treatment of mice challenged with syngeneic Rous sarcoma tumor cells.

Progress: A protein-associated component (about 60,000 daltons) has been isolated from protoplast membranes of streptococci. Three nanograms of this material can depress the antibody response of mice to sheep erythrocytes. The membrane preparations selectively suppress immature B cells both in vivo and in vitro. In contrast, certain T-cell functions are enhanced by exposure of cells to membrane material. A soluble suppressant can be detected in the supernate from cultures of bone marrow cells exposed to the membrane preparations. Removing T cells with anti-Thy 1.2 serum, or removing adherent cells, does not affect the capacity of streptococcal membranes to suppress B-cell responses. This suggests that we have demonstrated a B-cell regulatory factor produced by immature B cells.

Significance to Cancer Research: These studies support the concept that microorganisms, common in the human environment, can modulate the immune system. Thus, the indigenous or transient microflora could be a factor in determining "spontaneous" tumor regression or growth. Immunosuppressive or potentiating components isolated from bacteria are potentially useful as therapeutic agents.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Dr. Michael J. Embleton
Name/Address University of Nottingham
Performing Organization: Nottingham, England

Contract Number: NO1-CB-64009
Starting Date: 6/18/76 Expiration Date: 6/17/77

Goal: To determine (a) the frequency of expression of tumor-associated antigens capable of inducing host resistance in spontaneously arising tumors of an inbred rat strain, and (b) the influence of these antigens on tumor development.

Approach: Spontaneous tumors have been transplanted into syngeneic rats as they arose in a normal breeding population. Immunogenicity is assessed at early transplant generations by immunization/challenge protection studies in vivo. This is supported by studies of concomitant immunity and adoptive transfer of immunity in vivo, and in vitro assays for cell-mediated and antibody-mediated tumor immunity.

Progress: Previous experiments with a series of spontaneous mammary carcinomas, a squamous cell carcinoma, and a reticulum cell sarcoma have indicated that about a third of them were capable of inducing low levels of tumor immunity in syngeneic hosts, but most were apparently nonimmunogenic. Most of the tumors studied, including those against which no immunity could be induced, were characterized by fetal antigens detectable by in vitro tests. In the current period inbred rats have been immunized against a series of six spontaneous fibrosarcomas, and half of these have induced some degree of immunity to challenge with tumor cell suspensions. In the case of two sarcomas, resistance against an inoculum of 10^5 or more cells has been achieved, and a strong humoral antibody response has been detected against one of these using a membrane immunofluorescence test. Tumor cell challenges have been given by intravenous, intraperitoneal and subcutaneous routes, and up to the present the latter two routes have proven the most sensitive.

Significance to Cancer Research: Spontaneously arising animal tumors are more similar to those arising in man than are tumors induced artificially by chemical carcinogens or oncogenic viruses, and an analysis of their immunobiology will be highly pertinent to the immunology of human tumors.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components

Principal Investigator: Dr. E. D. Kiehn
Name/Address University of Washington
Performing Organization: Seattle, WA

Contract Number: NO1-CB-43987
Starting Date: 6/1/75 Expiration Date: 5/31/77

Goal: The clarification of the relationships between cell surface changes and the unrestrained growth patterns of malignant cells.

Approach: The basic approach has been to employ a wide array of cloned cell types in cell culture. Standard criteria have been used to characterize these cells. With special emphasis on conditional (thermosensitive) transformants, the mechanisms of transformation have been investigated.

Progress: Initially, numerous properties of normal polyoma-transformed BHK cells were examined. A number of aspects of the cell surface were dramatically thermosensitive in ts3-BHK cells. However, there were few if any coordinate changes in growth behavior or in other properties of transformation. In particular, cyclic nucleotides, plasminogen activator, and other proteases did not appear to be involved in these membrane alterations. Recently, clones of normal diploid rat embryo fibroblasts were transformed by SV40 and polyoma viruses. While extensive karyotypic alterations did not accompany transformation, dramatic changes in serum requirements, saturation density, anchorage dependence, and morphology were observed. Both viral and spontaneous transformants secreted less plasminogen activator than did the normal parent clones. Plasminogen activation did not appear to play any role in the growth and morphology of these normal and transformed rat clones. The secretion of plasminogen activator was reduced in normal cells rendered quiescent by serum limitation but did not appear to be regulated by the state of anchorage of either normal or transformed cells. Finally, the presence of exogenous plasmin was not a sufficient condition to initiate anchorage-independent cell division in the normal clones of rat fibroblasts. Fundamental properties of malignant cells depend on both the nature of the transforming agent and on the original cell type involved.

Significance to Cancer Research: A detailed molecular understanding of the properties of malignant cells may provide a practical basis for improvements in cancer detection, diagnosis, therapy, and prevention.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. Oliver Smithies
Name/Address University of Wisconsin
Performing Organization: Madison, WI

Contract Number: NO1-CB-43986
Starting Date: 6/28/74 Expiration Date: 6/27/77

Goal: To develop methods suitable for amino acid sequence determination of normal and tumor-associated antigens available only in radioactive tracer amounts.

Approach: To scale down the amount of material required for sequence analysis by using sequencing procedures dependent upon radioisotopes incorporated into the antigens during a brief time in small-scale cultures. The aim has been to label uniformly all the amino acids in the proteins at the same time, so that complete amino acid sequences can be obtained from a single sequence run. The test system used employs the normal human transplantation antigens (HLA).

Progress: First labeling procedures were developed adequate for the incorporation of ^{14}C amino acids into cells in tissue cultures in sufficient quantities to permit radioactive sequencing of the protein β_2-microglobulin which is released into the medium. Then methods were developed for isolating HLA antigens from the membranes of the same cells, using (a) a simple solubilization step with Triton X-100, (b) Sepharose beads coated with antiβ_2m, and (c) an analytical SDS gel electrophoresis run to permit the separation of the HLA from the β_2m. About 26 positions of the amino terminal sequence of HLA antigens isolated from 40 ml cultures were determined. The sequencing method is now applied to the internal parts of these antigens. Methods have been devised of purifying and sequencing the tryptic peptides of radioactive HLA antigens. Two purified, intrinsically labeled peptides have been completely sequenced.

Significance to Cancer Research: The method provides a means of studying the structures of any cancer-related antigen which can be isolated by a specific (immuno) adsorbant from cells able to be labeled in tissue culture.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Subpopulation of Specific Sera

Principal Investigator: Dr. Patricia Byfield
Name/Address The Upjohn Company
Performing Organization: Kalamazoo, MI

Contract Number: NO1-CB-64027
Starting Date: 4/12/76 Expiration Date: 9/30/76

Goal: Evaluation of a new method for preparing antisera which will react with lymphocyte subclasses.

Approach: The sera were prepared by immunizing chickens with shark spleen cells. They were then tested for their reaction with mouse lymphocytes, in the hope of finding antigens which had been conserved during evolution, in anticipation that some of these antigens might appear only on a "primitive" type of lymphocyte in the mouse.

Progress: Approximately 50 sera have been prepared and evaluated. The chicken anti-shark spleen sera do react with mouse lymphocytes. Some of the antigens occur in shark hematopoietic tissue and mouse lymphocytes, but not in other mouse and shark tissues. Preliminary work has been done to identify the specific cells and molecules with which these sera react. (This contract has now expired.)

Significance to Cancer Research: Identification and separation of lymphocyte subclasses are important as means to understanding their potential interactions with each other and with malignant cells. In mice, helper, suppressor, and killer cells can be distinguished by the antigens on their surfaces. The antibodies used to identify these cells are produced by immunizing inbred mice with cells from other inbred mice. This cannot be done in man, so a different approach is needed. We hope that this "immunophylogenetic" approach will succeed.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Hans Wigzell
Name/Address
Performing Organization: Uppsala University
Uppsala, Sweden

Contract Number: NO1-CB-64033
Starting Date: 6/28/76 Expiration Date: 6/27/77

Goal: To achieve information allowing us to understand the ways through which the immune system will react against tumor-associated antigens in syngeneic tumor systems. Subsequently, try to manipulate the immune system in ways allowing an increased reactivity toward the autochthonous tumor.

Approach: Using specific fractionation procedures, select subgroups of cells expected to participate in immune reactions against syngeneic tumors will be purified. Such "pure" subgroups of cells will then be analyzed when obtained either from normal or tumor-immune individuals as to the specific immune activity in vivo or in vitro.

Progress: Substantial progress has already been made in one area of this field, that of analysis of the s.c. "natural killer" cells existing in normal individuals and with relatively specific cytolytic effects on syngeneic T lymphomas. It was demonstrated that this cell type is of non-conventional type, originating from bone marrow in an autonomous manner (independent of thymus or spleen), but quite susceptible to interference with radioactive strontium treatment. Using chimeras it has been possible to produce individuals with high or low "natural killer" cell levels, and it is now clear that in vivo there exists a positive correlation between the resistance of the individual to transplantation of syngeneic leukemias and natural killer cell levels. In fact, under most conditions using T lymphomas it would seem clear that natural killer cells are a most decisive cell group with regard to tumor resistance with T and/or B lymphocytes playing insignificant roles.

Significance to Cancer Research: Any further understanding of the cell types involved in reactions to syngeneic tumor-associated surface structures should be of value if ever positive manipulation of the tumor-immune reactions will be possible in a predictable manner.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Granulocyte and Macrophage Proliferation Control in Leukemia and Related Neoplasms

Principal Investigator: Dr. Donald Metcalf
Name/Address
Performing Organization: Walter and Eliza Hall Institute
Victoria, Australia

Contract Number: NO1-CB-33854
Starting Date: 6/20/73 Expiration Date: 10/19/77

Goal: To chemically characterize and purify the glycoprotein, colony-stimulating factor (GM-CSF) controlling the proliferation of normal and leukemic granulocytes and macrophages, and to analyze its mechanism of action.

Approach: Two source materials have been used: mouse lung for mouse GM-CSF and human placenta for human GM-CSF. Standard procedures of separative protein chemistry have been used and all bioassays made using granulocyte and macrophage colony formation in agar cultures of normal or leukemic marrow cells.

Progress: In the past year, a regular production of pure mouse GM-CSF has been achieved. Methods have been developed for radiolabeling the molecule and initial studies have been made on membrane receptors. Human placental GM-CSF has been purified 1000-fold, has been field-tested, and is now widely accepted as the standard method for stimulating human marrow cultures, replacing previous unsatisfactory underlayers. Good progress has also been made in purifying the specific glycoproteins in pokeweed mitogen spleen-conditioned medium stimulating eosinophil and megakaryocyte proliferation.

Significance to Cancer Research: This work is providing specific monitoring systems of proven practical value for various hematopoietic populations in patients under therapy with leukemia and cancer. As all myeloid leukemias in man have been shown to be GM-CSF dependent, an alternative form of therapy may be feasible.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $137,479

CONTRACT RESEARCH SUMMARY

Title: Transfer Factor and Delayed Type Hypersensitivity in the Mouse

Principal Investigator: Dr. J.F.A.P. Miller
Name/Address Walter and Eliza Hall Institute
Performing Organization: Victoria, Australia

Contract Number: NO1-CB-63992
Starting Date: 3/1/76 Expiration Date: 2/28/78

Goal: To determine whether a state of specific cellular immunity can be conferred on naive (nonsensitized) mice by subcellular material extracted from lymphoid cells of appropriately sensitized mice. Such transfer is possible in humans and the active principle has been termed "transfer factor" (TF). The lack of a suitable animal model has been a major obstacle in characterizing TF chemically and functionally.

Approach: An objective and sensitive radioisotopic method was developed to measure delayed type hypersensitivity (DTH) in mice and characterize the genetic factors influencing successful activation of those T lymphocytes responsible for DTH induction and transfer. The contractor is now preparing subcellular material from lymphoid cells of sensitized mice and examining ability of this material to transfer specific sensitivity to naive mice.

Progress: Before any serious attempt can be made to prepare and evaluate TF in mice, certain fundamental aspects of DTH had to be examined. Highly specific DTH responses in mice could be transferred by T lymphocytes. Certain T-cell subsets were responsible for induction and transfer, whereas others, sensitive to cyclophosphamide, were involved in suppressing DTH. Certain genes in the major histocompatibility complex (MHC) on chromosome 17 influenced DTH responsiveness and transfer. Subcellular extracts of relevant T cells, prepared by methods analogous to those used for human TF, failed to transfer DTH in mice. Material released from antigen-pulsed macrophages (cells which process and present antigen to T cells), containing both an antigen moiety and an MHC-derived component, induced potent de novo sensitization of T cells. Possible relationship to TF is being studied.

Significance to Cancer Research: In many tumors, cellular immunity operates to retard tumor growth, whereas antibody may act as blocking factor. Because of its overall selectivity on T cell-mediated responses, TF-like material may be of great value in tumor immunotherapy, allowing a more effective T cell participation in DTH or cytotoxic reactions, and not augmenting blocking antibody.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $44,320

CONTRACT RESEARCH SUMMARY

Title: The Role of T and B Lymphocytes in Tumor Immunity

Principal Investigator: Dr. G. J. V. Nossal
Name/Address Walter and Eliza Hall Institute
Performing Organization: Victoria, Australia

Contract Number: NO1-CB-23889
Starting Date: 10/30/74 Expiration Date: 10/29/77

Goal: To study the development of T lymphocytes, analyze tumor immunity induced in vitro, and determine the mechanisms by which antigens and antigen/antibody complexes influence the immunological activities of lymphocytes.

Approach: Organ cultures of 14-day murine fetal whole thymus lobes, with assays of the immunological properties of the cultured thymus cells; in vitro induction of cytotoxic T lymphocytes directed against tumor-associated antigens with analysis of the specificity and genetic control of the immunity induced; and effect of anti-" " serum on T-dependent and T-independent immune responses.

Progress: Two peaks of thymus cell proliferation have been identified in the cultured fetal thymus. Cells from the first peak are hydrocortisone sensitive, Thy-1.2 and TL positive, show strong nonspecific suppressor activity, and are not responsive to PHA or Con A. The second peak cells are also Thy-1.2 positive but are now largely TL negative and hydrocortisone resistant, show less suppressive activity than the first peak cells, and are readily stimulated by Con A and PHA. Both H-2 linked and non-H-2 linked genetic control of the immune response to particular types of tumor-associated antigens have been identified. Immunofluorescent studies have shown that B cells of peripheral lymphoid organs bear both IgM and IgD, but in the bone marrow half of the B cells bear only IgM, suggesting that IgD appears later in ontogeny than IgM.

Significance to Cancer Research: With numerous immunological parallels between the persistence of the mammalian fetal allograft and a malignant tumor, the finding of T cells with marked suppressive effects in fetal thymus cultures raises the possibility that the fetus may play an active role in suppressing maternal rejection. The T lymphocyte is thought to play a major role in the immunosurveillance of mammalian neoplasia, so demonstration of genetic control of the T-cell response to tumor antigens bears directly on this question of the role of the genome in the susceptibility to cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $144,940

CONTRACT RESEARCH SUMMARY

Title: Detection and Development of H-2 Recombinant and Mutant Strains

Principal Investigator: Dr. Donald D. Shreffler
Name/Address Washington University
Performing Organization: St. Louis, MO

Contracting Number: NO1-CB-74154
Starting Date: 3/1/77 Expiration Date: 2/28/78

Goal: The primary goal of this project is the detection and fixation in congenic inbred strains of new recombinant and mutant H-2 haplotypes, most particularly those involving the I (immune response) region of the H-2 gene complex.

Approach: Several approaches are being employed, all of which employ the screening of large numbers of progeny from crosses involving different H-2 haplotypes: (1) testing of mutagens that may enhance the frequencies of recombination and mutation; (2) screening of progeny of newly arisen recombinants for enhanced frequencies of second-step recombination events; and (3) development of MLR and Ia serotyping methods for improved detection of I region mutants.

Progress: This project was only recently funded, so it is just being fully developed. All necessary crosses for the first stage of screening have been made and progeny are available for typing. The testing of improved MLR methods for more sensitive screening for I region mutations is already under way. One putative H-2 recombinant has been detected and is being progeny-tested.

Significance to Cancer Research: This program has two primary points of relevance to cancer research: (1) The I region of the H-2 complex plays a critical role in susceptibility to oncogenic viruses. The intra-H-2 recombinants and mutants produced in this program will contribute to the definition and characterization of the genes of the I region that control such susceptibility. (2) The serologically detected gene products of the K, D and I regions of the H-2 complex are directly involved in cell-mediated cytotoxicity reactions against neoplastic and virus-infected cells. The recombinants and mutants detected by this program will provide highly useful material for the chemical and functional characterization of these products.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $72,804

CONTRACT RESEARCH SUMMARY

Title: Studies of the Mechanism by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Dr. Irun Cohen
Name/Address Weizmann Institute
Performing Organization: Rehovot, Israel

Contract Number: NO1-CB-63982
Starting Date: 9/1/75 Expiration Date: 8/31/77

Goal: To learn how tumors influence the function of the immune system so that the tumor cells avoid destruction and enjoy enhanced growth.

Approach: Mice bearing tumors develop lymphocytes in their spleens which enhance the growth of tumor cells transplanted to other mice. This enhancement is studied by measuring the effects on immune functions of tumor-enhancing spleen cells or of enhancing factor (EF) secreted by such cells.

Progress: Progress was made during the past year in identifying (a) the cells of tumor-bearing mice (TBM) which produce the enhancing factor, and (b) aspects of the host immune response affected by the enhancing factor. EF was obtained by injecting C57BL/7 mice with the syngeneic 3LL tumor. About 16-18 days later, suspensions of spleen cells of the TBM were incubated in vitro and the cell-free medium was tested for enhancing activity by injecting syngeneic mice with 3LL tumor cells. EF was assayed by measuring enhancement of tumor growth produced by test medium compared to that produced by control medium. In addition to enhancing the growth of a variety of mouse tumors, injection of EF markedly increased the production of antibodies of both IgG and IgM types. EF appeared to be produced by cells which were not mature T lymphocytes, and EF facilitated the induction of antibody production. Therefore, enhancement of tumor growth might result from the production of blocking antibodies to tumor-cell antigens. EF appeared to be unlike previously described factors and may be a new factor.

Significance to Cancer Research: Study of the mechanisms by which tumors avoid immune destruction could indicate how to control them; the immune response of some cancer patients might be directed to attack and rid the patients of cancer cells.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $80,265 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator: Dr. Michael Feldman
Name/Address Weizmann Institute
Performing Organization: Rehovot, Israel

Contract Number: NO1-CB-63979
Starting Date: 9/1/75 Expiration Date: 8/31/77

Goal: To develop methods for in vitro sensitization of lymphocytes against autologous human malignant gastrointestinal tumors.

Approach: The contractor will seek optimal culture conditions for human tumor cells. Sensitization will be tested as a function of procedures for purification of lymphocytes, exposure of cell receptors to antigens, and immunopotentiation by adjuvants.

Progress: C57BL lymphocytes sensitized on monolayers of the syngeneic 3LL Lewis lung carcinoma developed specific cytotoxicity which was assayed by measuring the reduction in uptake of ^{3}H-leucine, ^{3}H-thymidine, or ^{51}Cr by target cells, following interaction with the sensitized lymphocytes. This method was useful for measuring cytotoxicity of human lymphocytes sensitized in culture against human tumor cells. High levels of cytotoxicity were obtained using peripheral blood lymphocytes sensitized on monolayers of human renal adenocarcinoma. Pretreatment of lymphocytes with poly-AU increased significantly the production of cytotoxic lymphocytes following in vitro sensitization on monolayers of tumor cells. When lymphocytes capable of binding to histamine-RSA-sepharose from a population of lymphocytes sensitized in culture against a syngeneic tumor were eliminated, a specific increase in anti-tumor cytotoxicity resulted. Lymphocytes, sensitized on monolayers of macrophages which had been fed with membranes containing cell-free material of tumor cells, developed specific anti-tumor cytotoxicity.

Significance to Cancer Research: NCP Objective 6 Approach 4

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $81,375 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Studies of the Mechanisms by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Dr. Nechama Haran-Ghera
Name/Address Weizmann Institute
Performing Organization: Rehovot, Israel

Contract Number: NO1-CB-43930
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: Analysis of the immunological tumor-host relationships during the latent period of leukemia induction involving the mechanism by which preleukemic cells escape destruction and the transition from a controlled proliferation of preleukemic cells to overt leukemia development.

Approach: Phases in leukemogenesis induced in C57BL/6 mice were studied: Identification and characterization of preleukemic cells and the tumor cell-host relationship induced by them expressed in build up of immunity, in comparison to overt neoplasia; the ultimate breakdown of immunity by tumor cell products or due to the shift from the "dependent" preleukemic variant to the "autonomous" leukemic cells.

Progress: Preleukemic cells could be identified among the bone marrow cells within 7-30 days following the different leukemogenic treatments irrespective of the ultimate high, or low overt leukemic incidence. Qualitative rather than quantitative differences exist between the preleukemic cells and the leukemic cells. Two fundamental phases were shown to occur during the long latent period: (1) neoplastic transformation induced by the different leukemogenic agents, expressed as preleukemic cells, and (2) differentiation and proliferation of the preleukemic cells, affected by host factors, to overt disease. Two stages were observed during the "proliferation" phase -- the "dependent" stage afforded by specific host environment (irradiated host) and the "autonomous" phase subdivided into "autonomous responsive" and "complete autonomy." The most unique recent findings indicated that preleukemic cells were capable to induce build up of immunity expressed by specific effector cells (identified as B cells) and specific antibodies induced by them in contrast to the leukemic cells which have no immunizing capacity (unless irradiated). Thus, the shift from the preleukemic phase that prevails during most of the long latent period to the sudden autonomous phase introduces changes in tumor-cell host relationship, thereby contributing to the escape phenomenon.

Significance to Cancer Research: These studies have provided information on host immune responses during early stages of tumor development and have contributed to analyzing the dilemma as to why preleukemic cells exist despite immune pressure.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Purified Thymic Products Promoting Lymphocyte Differentiation

Principal Investigator: Dr. Nathan Trainin
Name/Address Weizmann Institute
Performing Organization: Rehovot, Israel

Contract Number: NO1-CB-53926
Starting Date: 6/16/75 Expiration Date: 6/15/77

Goal: To chemically characterize thymic products or other antigens which have been shown in well-defined in vitro assays to promote differentiation of lymphoid cells from precursors.

Approach: Isolation, purification and characterization of thymus hormone as well as elucidation of its antigenic properties. Possible organic synthesis.

Progress: The contractor has demonstrated that THF, a thymic factor he has purified, induces immune maturation of lymphoid cells via an obligatory rise in membranal adenyl cyclase activity, and in intracellular levels of cAMP. The hormonal nature of THF has been established. These biological events are exerted only on thymus and thymus-derived cells (T cells). They have developed a procedure for isolation and characterization of the active principal of calf thymus. The active principal of THF is a polypeptide of molecular weight approximately 3000. There is a high proportion of acedic residues such as glutamine and aspartic acids on amino acid analysis. Future progress should include production and purification of THF on a larger scale to elucidate its amino acid sequence and establish an adequate radioimmunoassay.

Significance to Cancer Research: (NCP Objective 6 Approach 4
Objective 3 Approach 4)
Investigations of alterations in host immunologic responsiveness is an essential component of the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Dates: 8/21-23/74
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. D. C. Edwards
Name/Address Wellcome Research Laboratories
Performing Organization: Beckenham, Kent, England

Contract Number: NO1-CB-43929
Starting Date: 6/20/74 Expiration Date: 6/19/77

Goal: Substitution of the peptides containing the cell combining sites of diphtheria toxin and ricin by half molecules of specific antibody, so as to produce molecules containing the toxic moieties of these proteins conjugated via essential S-S linkage (as in the native molecules) to a new and chosen "address" moiety.

Approach: To prepare purified diphtheria toxin and ricin, and to isolate the toxin peptides. To prepare half molecules of normal IgG and of rabbit antibody against mouse lymphoma and Krebs ascites tumor and to work out conditions for conjugating these by S-S bonds to the toxic peptides. To set up a cell-free protein synthesis system (from rabbit reticulocytes) for assay of the toxic peptides and newly prepared conjugates and an assay for selective cell killing by the conjugates.

Progress: Half molecules of IgG and of rabbit antibody have been conjugated with diphtheria toxic peptide in reasonable yield, using reversible sulphonation of the free SH groups. The conjugate retained toxicity in the cell-free protein synthesis assay, but could not be tested on living cells because of difficulty in removing SDS completely. Preparation and separation of conjugates in the absence of SDS proved unexpectedly difficult, but conjugates with anti-lymphoma antibody are now ready for testing. Preliminary results suggest that ricin peptide (which is 20 times more potent than diphtheria peptide in the cell-free assay) may need to be conjugated by a different method.

Significance to Cancer Research: Our hope is to prepare an antibody-directed "magic bullet" using anti-tumor antibodies. We cannot yet be certain that any conjugates will avoid being ingested by macrophages.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. Richard K. Gershon
Name/Address Yale University School of Medicine
Performing Organization: New Haven, CT

Contract Number: NO1-CB-43994
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To identify populations with the ability to suppress the response of other lymphoid cells to the same and/or different antigens after transfer in vitro or to syngeneic hosts. Classification of suppressor cell types and study of factors responsible for the generation of suppressor T cells.

Approach: Study the effect of cyclophosphamide (CY) on delayed type hypersensitivity (DTH) to cell surface antigens and the effect of histamine on in vitro cell-mediated immune responses. Using a system involving in vitro antibody responses, vary antigen dose, timing before transfer and use of antisera to T-cell subpopulations to trace cell interactions important for suppressor T-cell generation.

Progress: CY treatment prior to immunization often results in augmentation of DTH, implying an effect on a CY-sensitive suppressor cell population. The existence of a CY-sensitive bidirectional regulatory T-cell population has been demonstrated. The direction of regulation depends on the underlying level of the immune response and to a lesser extent on the antigen or assay tested. In recent studies it was shown that the vasoactive amine histamine inhibits in vitro cell-mediated immune reactions. Preliminary evidence shows that the mechanism of inhibition is by generation of regulatory T cells. Using an in vitro antibody generating system, optimal suppressor cell activity has been shown to be time and antigen dose dependent. Several loops of T-cell interaction occur during the induction and effector phases of suppressor cell activity. Helper cells can turn on fully differentiated suppressor T cells and also induce less mature uncommitted T1 type T cells to act in a suppressive fashion.

Significance to Cancer Research: The histamine receptor may be a trigger for a "second signal" in suppressor T cells or their precursors. Therefore, manipulation of cells through these receptors may modify suppression existent during the response to tumors. Using surface receptors as tools, selective depletion of suppressor T cells may gain primary importance as a future immunotherapeutic tool against human cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection of Antigen-Binding Activity of Transplantable T-Cell Tumors

Principal Investigator: Dr. Frank F. Richards
Name/Address Yale University School of Medicine
Performing Organization: New Haven, CT

Contract Number: NO1-CB-63993
Starting Date: 9/1/75 Expiration Date: 8/31/77

Goal: Detect antigen-binding receptors on T-cell tumor lines.

Approach: (1) Screen supernatant solutions in which T-cell tumor cell lines have been grown for protein components capable of binding antigens. (2) Use of the bubble technique to test for direct binding of antigen to T cells.

Progress: So far five tumor cell lines have been screened against a bank of 400 antigens with negative results. Two further cell lines will be tested during the spring and early summer of this year. These are WEHI 22 and WEHI 7, two "T-cell" tumor lines which are known to have immunoglobulin-like structures attached to their cell surface. The results so far bear out the findings of other workers in this field; i.e., that T-cell tumor lines apparently do not bear antigen-binding receptors on their surface.

Significance to Cancer Research: The search for T-cell receptors should be carried out on functional T cells, not on tumor lines.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $57,620 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Purified Thymic Products and Other Agents Promoting Lymphocyte Differentiation

Principal Investigator: Dr. Byron H. Waksman
Name/Address Yale University School of Medicine
Performing Organization: New Haven, CT

Contract Number: NO1-CB-43926
Starting Date: 6/17/74 Expiration Date: 11/15/77

Goal: Obtain and purify "lymphocyte activating factor" (LAF) from various types of macrophages and investigate kinetics of LAF production, improved conditions of production, and mechanism of LAF action on mouse and rat T cells.

Approach: Survey a variety of normal cell populations containing macrophages and continuous macrophage-like cell lines for production of LAF. Study various methods of culture, kinetics of LAF production, and techniques of purification. Test effect of LAF on different types of target lymphocytes and different parameters of response (macromolecular synthesis, lymphokine production, cytotoxicity) and on cell cycle. Use pharmacologic agents to analyze mechanism.

Progress: LAF or LAF-like activity (mitogenic for murine thymocytes) has been obtained from human and murine monocytes and from several adherent cell lines in culture: P-388 D1, J774-1, PU-5, WEHI-3. Activity has been demonstrated in molecules with molecular weights of ca. 13,000 and 80,000. At least 10 purification techniques have been evaluated. By use of hollow fiber devices, isoelectric focusing, and polyacrylamide gel electrophoresis, up to 1500-fold purified, active LAF can be obtained.

Rat macrophage-derived LAF has been shown to act on stimulated rat T cells only during the late G_1 phase of the cell cycle, to be insensitive to cyclic AMP level, but to depend on cAMP-sensitive events during the G_0-G_1 step of lymphocyte triggering.

Significance to Cancer Research: Macrophage products like LAF are essential participants in triggering the T lymphocytes responsible for specific immunity in cancer. A full knowledge of LAF's chemistry and biological action is needed to permit us to influence the induction of tumor immunity.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $70,199

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. Maria Linder
Name/Address: California State University
Performing Organization: Fullerton, CA

Contract Number: NO1-CB-74119
Starting Date: 11/1/76 Expiration Date: 10/31/77

Goal: Ceruloplasmin (CP) is a copper-containing α_2-glycoprotein of plasma with enzymatic activity. The activity and concentration of CP are elevated in animals with tumors and in humans with lung cancer. The goal of this contract is to determine whether this phenomenon prevails for humans with other kinds of cancer and whether tests of CP would be useful in early diagnosis, for prognosis, or in treatment of the disease.

Approach: The primary approach is to measure CP antigen concentration, enzyme activity, and plasma copper concentrations in large panels of sera from cancer patients and controls, examining different kinds of cancer, different stages of the disease, patients before and after treatment of recurrence, and, in the case of lung cancer, heavy smokers who may develop the disease.

Progress: In initial studies of lung cancer patients, a clear relationship between the degree of CP (and Cu) elevation and disease stage was noted: after successful therapy levels return to normal; after surgery, generally but not always, CP and Cu levels decrease; patients who develop a recurrence maintain higher than normal levels of CP after primary treatment. Irradiation and chemotherapy have different effects in different individuals, and further evaluation is required. Sera from breast cancer patients show even higher levels of CP than those with lung cancer in initial studies. In soluble extracts of normal and malignant human tissues immunological and enzymatic evidence was obtained for the presence of CP in amounts which could not be accounted for by residual blood. The possibility that cultured human lung tumor cells secrete CP into the medium is now being investigated.

Significance to Cancer Research: Potentially, this project is of great significance in that it may offer a chemical/immunological serum test for gauging cancer stage and size, a means of early diagnosis for several forms of cancer, and a way of judging the success of therapy, and following the course of the disease. It may also offer a fundamental insight into the metabolism of tumor cells.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 FUnds: $28,435

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-Associated Antigens

Principal Investigator: Dr. George L. Wright
Name/Address Eastern Virginia Medical Authority
Performing Organization: Norfolk, VA

Contract Number: NO1-CB-53955
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To isolate, purify, and identify human tumor-associated antigens.

Approach: Isolate and purify tumor-associated antigens from crude tumor extracts of urogenital tumors and test them for functional activity in assays of humoral and cell-mediated immunity.

Progress: Extracts prepared from renal cell carcinomas and other urogenital tumors were tested for antigenic activity using the leukocyte migration inhibition test. Many of the extracts tested demonstrated little activity or specificity. One renal carcinoma extract was found to be reactive and adequately discriminated between renal carcinoma patients, normal donors, and patients with other types of cancer. Sixty-three percent of the patients with renal carcinoma and only 7% of the normal donors and 30% of patients with other cancers were positive. The reactivity appeared specific since only 20% of the renal carcinoma patients reacted to a normal kidney extract. Additional testing showed that some renal carcinoma patients gave positive tests to a lung cancer extract; the nature of this cross-reactivity has yet to be determined. A tumor-associated antigen was partially purified from the crude renal carcinoma extract and was demonstrated to be slightly increased in both activity and specificity.

Significance to Cancer Research: The availability of purified and well-characterized tumor antigens would greatly facilitate the development of more sensitive immunodiagnostic tests for the screening and management of the cancer patient.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays for Circulating Tumor-Associated Antigens: CEA

Principal Investigator: Dr. Theodore Hersh
Name/Address
Performing Organization: Emory University
Atlanta, GA

Contract Number: NO1-CB-43887
Starting Date: 3/15/74 Expiration Date: 3/14/77

Goal: To determine the clinical usefulness of carcinoembryonic antigen (CEA) and other tumor-associated antigens, such as CEA-S, as adjuncts in the differential diagnosis of gastrointestinal cancer.

Approach: Patients with gastrointestinal symptoms had blood drawn for CEA level and other tumor-associated antigens. The clinical work-up was accomplished as is usual in order to arrive at a specific diagnosis. At the end of the year, the patients were clinically re-evaluated.

Progress: Over 1,600 patients have been enrolled. Blood has been sent to the reference laboratories for CEA and CEA-S assays. The patients were reevaluated at a year's anniversary to verify the initial diagnosis. Preliminary results are being obtained from the computerized data base on 1,156 patients enrolled in the study; 79% had an initial diagnosis of a benign condition, 13% had a malignancy, and in 8% no definitive diagnosis was established. There is a significant overlap between the CEA values of patients with initial benign and malignant diagnoses. Values found for CEA-S indicated that 96.8% of cases with benign disease were in the normal range, compared to 81.5% of those with cancer. For CEA, 10 of 872 (1.1%) of cases with benign diagnoses had values above 20 compared with 23 of 129 (17.8%) patients with malignancy.

Significance to Cancer Research: (Preliminary) There is a considerable overlap of normal and elevated CEA values in cases with benign and malignant conditions of the gastrointestinal tract. This lack of specificity does not make the CEA assay a useful adjunct in the differential diagnosis of cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Dates: 2/14/74; 12/2/75
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $8,508

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays of Circulating Tumor-Associated Antigens: AFP and HCG

Principal Investigator: Dr. Melvin Moore
Name/Address Emory University
Performing Organization: Atlanta, GA

Contract Number: NO1-CB-43888
Starting Date: 3/15/74 Expiration Date: 3/14/77

Goal: To evaluate the sensitivity and specificity of serum HCG and AFP values in the diagnosis of testicular masses. To correlate levels of HCG and AFP with disease activity in germinal testicular malignancies.

Approach: AFP and HCG values are followed serially in patients with known testicular tumors and correlated with disease activity, as well as prior to diagnosis or orchiectomy.

Progress: Prior to diagnosis, elevated markers were seen in 66% (4/6) of patients with non-seminomatous germinal tumors, 25% (2/8) of patients with seminoma, and 0% of patients (0/29) without germinal tumors. Marker studies were obtained in an additional 52 patients after orchiectomy. No elevations of HCG or AFP were seen in 4 patients with non-germinal tumors. Among patients with clinically active germinal tumors, 0% (0/2) of seminoma patients and 74% (14/19) of non-seminoma patients had elevated HCG or AFP levels. Among germinal tumor patients without clinically active disease 0% (0/8) of seminoma patients, and 11% (2/19) on non-seminoma patients had elevated HCG or AFP levels. In this series of patients there were no false positive pre-operative elevations of AFP and HCG in patients with testicular masses. Elevations of AFP or HCG in the absence of clinical disease were seen in 7% (2/27) of patients with prior history of germinal tumors and in 0% (0/34) of patients with no prior history of germinal tumors. Among patients with active germinal malignancy, 28% of those with non-seminomatous tumors had falsely negative normal markers as compared with 80% of those with seminoma. The clinical course closely paralleled fluctuations in HCG or AFP in 20/22 patients with elevated marker levels.

Significance to Cancer Research: The tumor markers HCG and AFP have been shown to be quite specific in the diagnosis of germinal tumors of the testes in the young male patient presenting with a testicular mass. The clinical utility of following HCG and AFP serially in patients with non-seminomatous testicular tumors has also been demonstrated. Marker studies are rarely abnormal in patients with seminoma.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 12/2/76
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Tumor-Specific Antigens in Diagnosis and in the Management of Cancer Patients

Principal Investigator: Dr. T. M. Chu
Name/Address Health Research, Inc.
Performing Organization: Roswell Park Memorial Institute
Buffalo, NY

Contract Number: NO1-CB-33858
Starting Date: 6/25/73 Expiration Date: 10/24/77

Goal: (1) Evaluate the prognostic value of the preoperative CEA relating to disease recurrence or progression; (2) compare the prognostic value of CEA to other major prognostic parameters of colorectal cancer, such as Dukes' classification; (3) develop a predictive model which integrates the CEA levels with other major prognostic variables to determine the probability of recurrence or progression; and (4) implement a clinical plan for patients whose risk of recurrence is greatest based upon preoperative and follow-up CEA measurements.

Approach: This will be done by determining a pretreatment CEA and serial postoperative CEA 4 and 14 days after surgery, and as well as 1 and 2 months and every 3 months thereafter. The patient's clinical information is closely observed and recorded for clinicopathological correlation. In addition, several statistical analyses are performed. Finally, a predictive model will be developed and evaluated clinically.

Progress: A long-term follow-up of prognostic values of the CEA has been studied in 74 patients with resectable colorectal cancer for 12-60 months. A total of 19 developed recurrence of disease. The median time to recurrence was 24.1 months, with the first recurrence at 7.1 months. Fourteen patients died, with the first death occurring at 8.6 months (median time, 39.0 months). Plasma CEA was found to be independent of age, sex, cell differentiation, or site of tumor. A discrete data analysis indicated that the preoperative CEA is a significant indicator of disease recurrence. Based upon various contingency table analyses and tests on a linear logistic model, no significant correlation was shown between CEA and Dukes' classification. However, the combination of CEA and Dukes' was more significant (sensitive) as a prognostic parameter than either indicator alone. Patients with Duke C tumor and an elevated preoperative CEA (>2.5 ng/ml) had the worst prognosis.

Significance to Cancer Research: CEA is a laboratory assay which can be used as an aid in the management of certain cancer patients.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $70,586

CONTRACT RESEARCH SUMMARY

Title: Development of Macrophage Electrophoretic Mobility Assay for Malignant Disease

Principal Investigator: Dr. Leonard Weiss
Name/Address Health Research, Inc.
Performing Organization: Roswell Park Memorial Institute
Buffalo, NY

Contract Number: NO1-CB-53901
Starting Date: 6/1/75 Expiration Date: 5/31/77

Goal: Investigation of the macrophage electrophoretic mobility assay as a valid tool in the diagnosis and monitoring of malignant disease.

Approach: Establishment of the assay and refinement of cell preparation and electrophoresis techniques. Preparation of data in such a way as to allow direct comparison of results among independent investigators. Examination of series of patients with various clinically confirmed and staged cancer, including premalignant lesions, together with appropriate controls. Exploration of the biologic basis of the assay using experimental animals bearing tumors or challenged with conventional antigens. Performance of the assay on a "blind" trial basis with coded specimens organized by NCI.

Progress: The assay has been refined and performed on a series of patients with clinically confirmed colorectal cancer and controls. The test gave positive results in a high percentage of patients, but approximately 30% of apparently healthy controls also gave positive results. A series of patients with melanoma is currently being examined with positive results in a large majority of cases. A second series of assays on patients with premalignant and malignant skin lesions during the course of their treatment has been initiated. Patients with a history of cutaneous malignancy, where progress following treatment is currently being monitored, are also being examined and greater than 95% gave positive results. "Blind" trial studies with coded specimens organized through NCI have been started. Preliminary studies with experimental animal model systems have so far only indicated the possibility of a more universal biologic application of the assay.

Significance to Cancer Research: This assay could be potentially useful in monitoring the clinical status of patients with, or with a history of, cancer rather than in initial detection of the disease. It could also provide a method of examining the apparently important role played by macrophage-lymphocyte-tumor interactions in the progress of malignant disease.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: New In Vitro Techniques to Evaluate Cell-Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing Organization: Baltimore, MD

Contract Number: NO1-CB-43965
Starting Date: 5/16/74 Expiration Date: 5/15/77

Goal: This contract has sought ways in which cell-mediated cytotoxic assays could be adapted to enable detection of cell-mediated immunity against syngeneic tumor.

Approach: Low molecular weight markers have been used to detect minimal membrane permeability changes induced in target cells by lymphocytes. It was hypothesized that by measuring such minimal damage one might be able to increase the sensitivity of cytotoxic assays in vitro. Attempts have been made to increase the lytic activity of lymphocytes by culturing effector cell populations under varying conditions before assay.

Progress: Although it could readily be shown that ^{86}Rb flux preceded ^{51}Cr release in cell-mediated lytic assays, we found no evidence that use of the low molecular weight marker increased the sensitivity of the assay. Lesions that initially allow ^{86}Rb efflux do not anneal, but enlarge (probably by colloid osmotic pressures) and eventually allow ^{51}Cr release and disruption of the cell membrane. Culturing mouse effector lymphocytes in vitro even for fairly brief (16 hours) periods led to a marked increase in lytic activity. Considerable evidence was gathered to support the view that the augmented lytic activity was due to differentiation of new killer cells in culture. These findings enabled demonstration of killer cells against a variety of syngeneic tumors in situations where no such reactivity was demonstrable before culture.

Additionally, novel methods of calculating the incidence of killer cells in lymphocyte populations and of characterizing the affinity of effector cells for homologous target cells have been reported and allow a more quantitative appraisal of data generated in cell-mediated cytotoxic assays than had previously been possible.

Significance to Cancer Research: This contract was aimed at increasing the sensitivity and quantitativeness of a common in vitro cytotoxic assay. Such studies may eventually result in an assay system that would allow a patient's immunological status with respect to his tumor to be correlated with his clinical progress.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Groups: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-Associated Antigens

Principal Investigator: Dr. Calvin A. Saravis
Name/Address Mallory Institute of Pathology
Performing Organization: Boston, MA

Contract Number: NO1-CB-64071
Starting Date: 6/16/76 Expiration Date: 5/31/77

Goal: To develop an assay system for the tumor marker, ZGM, that is useful in the diagnosis and management of digestive tract malignancy.

Approach: To develop a sensitive radioimmunoassay for detecting ZGM in the blood of patients with gastrointestinal cancer; use of immunofluorescence microscopy to detect ZGM in surgical and biopsy specimens for histopathological diagnosis and prognosis; to correlate the results of the presence of ZGM in blood and its detection in histological sections with the presence or absence of malignancy.

Progress: ZGM was detected in primary and metastatic colon cancer and was not detected in normal human plasma, normal colon, normal liver, and normal spleen. It was distinct from CEA, from antigens cross-reacting with CEA, AFP, alpha-2 macroglobulin, ferritin-like molecules, blood group antigens, and buffy coat. During the present contract year purified ZGM and avid, high-titer anti-ZGM antisera were produced. A ZGM radioimmunoassay is being developed. Immunofluorescence microscopy was used for selecting the ZGM-positive tumors from which ZGM was isolated. Thirty-four of 39 colon adenocarcinomas, 6 of 6 gastric adenocarcinomas, and 0 of 20 non-G.I. carcinomas were ZGM positive. In addition, ZGM was detected in the lymphatic-containing tumor draining the primary tumor.

Significance to Cancer Research: The detection of the ZGM in patient specimens may permit earlier diagnosis, better evaluation of prognosis and monitoring of therapy for cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $91,071

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens of the GI Tract Other Than CEA and α-Fetoprotein

Principal Investigator: Dr. Elliot Alpert
Name/Address Massachusetts General Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-43889
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: The goal of this contract has been to search for new human tumor-associated antigens of the gastrointestinal tract other than carcinoembryonic antigen and alpha-fetoprotein.

Approach: The approach has been to render rabbits tolerant to normal plasma and tissue extracts and to immunize with cytoplasmic and membrane extracts as well as with intact tumor cells, in an attempt to raise heterologous antisera to tumor-specific antigens.

Progress: A total of 54 cancer tissues, from malignancies of the stomach, pancreas, colon and liver, were extracted in either saline or 3M KCl or were dissociated into isolated cell preparations. A total of 63 normal tissues were also collected and extracted or prepared as controls. Partial immune tolerance to normal human serum, plasma, and normal tissue antigens was induced in 71 rabbits. These rabbits were then immunized with cancer extracts or isolated cell preparations prepared from fresh human primary liver carcinoma and colon carcinoma tissue or cancer cell lines of human origin. Pools or antisera from the 50 rabbits have been completely absorbed to pools of normal human plasma, serum, and normal tissue extract antigens and tested against cancer tissue, membrane, or cell extracts. Cancer extract antigens absorbed were eluted and appeared as two distinct bands on polyacrylamide gel electrophoresis. Five antisera pools still contained precipitins to either KCl or PBS extracts of tumor tissue and appear to recognize tumor-specific antigens in two malignancies.

Significance to Cancer Research: There is increasing evidence that malignancies of the gastrointestinal tract contain tumor-specific antigens other than CEA and AFP. Our recent data suggest that heterologous antisera can be raised to these antigens. If specificity of these antisera to human tumor-specific antigens can be confirmed by further testing, it would enable isolation and characterization of these antigens, as well as the development of a new immunodiagnostic test for GI malignancy.

Project Officer: R. Raymond Gantt, M.D.
Program: Tumor Immunology Program Site Visit Date: 4/5/74
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Blood Sample Collection Bank as Reservoir for Evaluation of Cancer Detection Tests

Principal Investigator: Dr. Allan Schutt
Name/Address: Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-23879
Starting Date: 6/15/72 Expiration Date: 11/14/77

Goal: To establish and maintain a bank of sera from patients with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation of immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the Tumor Immunology-Immunodiagnosis Program.

Progress: The Mayo Serum Bank is a central storage area for a large number of blood samples, including those collected by Immunodiagnosis contractors at the Philadelphia Geriatric and the University of Minnesota cancer screening facilities aside from collection of samples. The Mayo Bank also ships panels of serum samples to investigators under the Immunodiagnosis Program's direction to evaluate potential immunodiagnostic tests for cancer in a blind fashion. Over 70 such test panels have been sent out in recent months. Extensive data are kept on all samples and continuous efforts are made to increase deficient areas of benign or malignant diseases.

Significance for Cancer Research: (NCP Objective 5 Approach 4)
This serum bank will provide sera, in sufficiently large quantities and with adequate controls, to efficiently and rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 2/19/74
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $100,458

CONTRACT RESEARCH SUMMARY

Title: Detection of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Dr. Fiorenzo Paronetto
Name/Address Mt. Sinai School of Medicine
Performing Organization: New York, NY

Contract Number: NO1-CB-43953
Starting Date: 5/16/74 Expiration Date: 5/15/77

Goal: Determine the feasibility of cancer diagnosis through the presence of antibodies against autologous tumors utilizing fluorescent antibody techniques.

Approach: Tumor tissues and sera from patients with tumors of colon and breast were tested for the presence of autoantibodies using the fluorescent antibody technique.

Progress: Utilizing fluorescent antibody techniques, the contractor has previously demonstrated in vitro complement fixation in 68% (21/31) of carcinomas of the colon. IgG autoantibodies against tumors were seen in only 4.5% (1/22) of sera of patients with carcinoma of the colon and 6.5% (2/31) of sera of patients with carcinoma of the breast. Utilizing cell suspension techniques, IgG autoantibodies were detected in sera of 20% (2/10) of patients with carcinoma of the colon. Fifty percent (2/4) of sera of normal controls likewise contained IgG antibodies against the carcinoma cell suspensions which were positive with autologous sera. Sixteen percent (3/19) of sera of patients with colon carcinoma and 16.5% (2/12) of sera of normal controls contained homologous antibodies against an established colon tumor line (HT-29). IgG antibodies active at 4°C (cold antibodies) against autologous carcinoma were detected in 83% (5/6) of sera of patients with carcinoma of the breast. Similar cold antibodies against homologous carcinoma of the breast were detected in 49% (18/37) of normal sera and 60% (22/37) of sera of patients with carcinoma of the breast. Strong cold antinuclear antibodies were detected in 44% (12/27) of sera of patients with carcinoma of the breast, but only in 11% (3/27) of sera of normal controls.

Significance to Cancer Research: The presence of antibodies against tumor in a large proportion of normal sera precludes the use of these antibodies for tumor diagnosis. The antibodies may, however, play a significant role in natural immunity against tumors.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 2/3/76
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-Associated Antigens

Principal Investigator: Dr. Arthur Malley
Name/Address
Performing Organization: Oregon Regional Primate Research Center
Beaverton, OR

Contract Number: NO1-CB-53954
Starting Date: 6/16/75 Expiration Date: 6/15/77

Goal: Isolate homogenous preparations of tumor-associated antigens from human melanoma and squamous cell carcinoma tissue in sufficient quantity to permit biological and chemical characterization.

Approach: Tumor tissue is extracted, purified, and tested for the presence of tumor-specific antigens by dermal skin tests and leukocyte adherence inhibition.

Progress: The optimal conditions were explored for the extraction of melanoma tumor-specific antigens from melanoma tissue. A wide variety of reagents were evaluated, and 4M KCl provided the maximum release of melanoma tumor antigens. An in vitro method (leukocyte adherence inhibition assay) was developed for detecting tumor-specific melanoma antigens. The specificity of the LAI assay was examined by testing various tumor extracts with leukocyte suspensions of tumor patients and normal healthy donors. A linear relationship exists between the concentration of protein in a melanoma extract giving a 10 mm indurated skin reaction and the concentration of protein giving a maximum LAI reaction. A melanoma antigen fraction was obtained containing two to six bands on polyacrylamide disc electrophoresis and a 2000-fold increase in tumor-specific melanoma antigen activity. Of interest is the observation suggesting that a relationship might exist between melanoma antigens and human β_2-microglobulin: significant amount of melanoma antigen activity was bound to a Sepharose-anti β_2-microglobulin adsorbent and eluted with 3M NaSCN. Further testing will involve the blocking of melanoma antigen binding to the adsorbent by pretreatment of the adsorbent with purified β_2-microglobulin.

Significance to Cancer Research: The availability of pure tumor antigens will provide a reagent that will permit the development of sensitive in vitro radioimmunoassays for the early detection of tumor antigen. In addition, the structural characterization of these pure tumor antigens may provide some insight on how tumor antigens escape the immune apparatus of the host.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 5/21/76
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Dr. Henry Altschuler
Name/Address Philadelphia Geriatric Center
Performing Organization: Philadelphia, PA

Contract Number: NO1-CB-33914
Starting Date: 6/25/73 Expiration Date: 10/24/77

Goal: To collect serial serum specimens from populations at high risk of contracting malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Demographically stable populations at high risk of developing human malignancies in the colon, lung, breast, prostate, bladder, pancreas, etc. will be followed on an annual basis. This includes clinical evaluation and serum collection. The sera will be banked and records kept on the patients so that information obtained from the stored sera can be correlated with development of any particular cancer.

Progress: Sera are being continually collected from a demographically stable population of high cancer risk aged residents of the Philadelphia Geriatric Center as well as from others. Complete records are being kept on each patient, including medical information and follow-up data from such sources as autopsies and Tumor Board Conferences. The collected sera are being sent to Mayo Clinic for storage under Dr. Schutt's project. As of February 28, 1977, a total of 2,816 serum samples were collected and 2,759 such samples were shipped. During this period there has been a total of 251 deaths, of which 41 were autopsied. In this same period 40 subjects have developed malignancies. Of these, 23% have G.I. cancers. In the order of decreasing frequencies are cancers of skin, breast, and prostate. In addition, there are a small number of miscellaneous malignancies. It should be pointed out that the population under survey is predominantly female.

Significance to Cancer Research: Early detection of cancer by means of appropriate testing would permit treatment of cancer at the most favorable time.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $52,768

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-Associated Antigens of the GI Tract Other Than CEA and Alpha-Fetoprotein

Principal Investigator: Dr. Peter H. Schur
Name/Address Robert B. Brigham Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-43958
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: The goal of this contract has been to search for new human tumor-associated antigens of the gastrointestinal tract other than carcinoembryonic antigen and alpha-fetoprotein.

Approach: The approach has been to render rabbits tolerant to normal plasma and tissue extracts and to immunize with cytoplasmic and membrane extracts as well as with intact tumor cells, in an attempt to raise heterologous antisera to tumor-specific antigens.

Progress: A total of 54 cancer tissues, from malignancies of the stomach, pancreas, colon and liver, were extracted in either saline or 3M KCl or were dissociated into isolated cell preparations. A total of 63 normal tissues were also collected and extracted or prepared as controls. Partial immune tolerance to normal human serum, plasma, and normal tissue antigens was induced in 71 rabbits. These rabbits were then immunized with cancer extracts or isolated cell preparations prepared from fresh human primary liver carcinoma and colon carcinoma tissue or cancer cell lines of human origin. Pools of antisera from the 50 rabbits have been completely absorbed to pools of normal human plasma, serum, and normal tissue extract antigens and tested against cancer tissue, membrane, or cell extracts. Cancer extract antigens absorbed were eluted and appeared as two distinct bands on polyacrylamide gel electrophoresis. Five antisera pools still contained precipitins to either KCl or PBS extracts of tumor tissue and appear to recognize tumor-specific antigens in two malignancies.

Significance to Cancer Research: There is increasing evidence that malignancies of the gastrointestinal tract contain tumor-specific antigens other than CEA and AFP. Our recent data suggest that heterologous antisera can be raised to these antigens. If specificity of these antisera to human tumor-specific antigens can be confirmed by further testing, it would enable isolation and characterization of these antigens, as well as the development of a new immunodiagnostic test for GI malignancy.

Project Officer: R. Raymond Gantt, M.D.
Program: Tumor Immunology Program Site Visit Date: 4/5/74
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of an Isomeric Species of Carcinoembryonic Antigen (CEA-S)

Principal Investigator: Dr. Robert M. Nakamura
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: NO1-CB-53934
Starting Date: 5/16/75 Expiration Date: 5/15/77

Goal: CEA-S is a specific isomeric species of carcinoembryonic antigen which is found predominantly in tumor tissues and can be detected in fetal and normal body fluids. The workscope of the contract is (1) to isolate and characterize CEA-S from colon carcinomas; (2) to produce specific antisera to CEA-S; and (3) develop an optimally specific and sensitive and standardized assay system for CEA-S.

Approach: General experimental approach to achieve these objectives is (1) isolation of the CEA-S ligands from several colonic carcinomas; (2) comparison of the ligands against a panel of sera with various diseases including gastrointestinal tumor, normal sera, inflammatory bowel disease, and renal diseases; (3) production of the antisera to CEA-S and conventional CEA; and (4) determination of the contribution of potentially interfering glycoproteins to the CEA-S assay such as normal cross-reacting glycoprotein (NCA).

Progress: (1) CEA-S has been isolated from a total of eight tumors; (2) the ligands have been compared against a panel of 65, including pools of normal gastrointestinal, other neoplasias, and renal inflammatory bowel diseases; (3) ten different antisera have been evaluated in the CEA-S assay and, based on diagnostic specificity, the optimal antiserum has been identified; (4) normal cross-reacting glycoproteins have been isolated and a specific radioimmunoassay developed; (5) for the past year panels of serum have been received from Drs. T. Hersh and Ming Chu (Emory University and Roswell Park, respectively), and these panels have been assayed and have been returned to NCI for evaluation.

Significance to Cancer Research: The serum CEA-S assay will be a more standardized assay which has greater specificity for gastrointestinal tumor than the conventional CEA assay. This CEA-S assay will definitely be more helpful in the evaluation of patients with bowel cancer and aid in the differential diagnosis of bowel cancer versus inflammatory bowel disease.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $121,005

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Soluble Human Tumor (CEA)-Specific Antigens

Principal Investigator: Dr. Ralph A. Reisfeld
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: NO1-CB-43277
Starting Date: 1/1/74 Expiration Date: 12/31/76

Goal: The purification of carcinoembryonic antigen (CEA) from tissue extracts and other fluids utilizing nondenaturing conditions, and the evaluation of these procedures as a means of producing highly specific radioimmunoassay (RIA) reagents.

Approach: CEA has been purified from saline extracts of tumor tissue by the use of lectin-affinity chromatography, molecular sieve chromatography, and isoelectric focusing; serum CEA has been isolated through the use of immunoadsorbents.

Progress: Classical purification procedures result in increased heterogeneity of the CEA population, as evaluated by lectin binding and by molecular sieve chromatography, due primarily to the use of lyophilization procedures for the purpose of concentrating samples. A procedure was developed for the immunization of animals with serum CEA, or with anti-CEA reactive substances derived from the sera of individuals with other malignancies. Unfortunately, the resulting antisera did not exhibit increased specificity, but the failure of immunoadsorbent-bound cross-reacting antigens to stimulate an anti-CEA response did suggest some differences in these antigens. By employing low concentrations of non-ionic detergents in the RIA incubation mixtures, it was shown that antigenic determinants lost during classical (denaturing) purification procedures could "regenerate," providing further evidence that the loss of antigenic activity after perchloric acid treatment and lyophilization is due to alterations in the antigenic structure of the molecule.

Significance to Cancer Research: If the reagents recently produced are shown to result in an assay of enhanced specificity and/or sensitivity, improved prognostic and diagnostic procedures will be developed, and a means of detecting gastrointestinal neoplasia may result.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-Associated Antigens

Principal Investigator: Dr. Ralph A. Reisfeld
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: NO1-CB-53899
Starting Date: 6/1/75 Expiration Date: 5/31/78

Goal: Development of new methods for the purification and physicochemical characterization of human tumor-associated antigens and to make them available for cell-mediated immunity assays and for diagnostic testing.

Approach: Human melanoma-associated antigens (MAA) are isolated by mild, least denaturing procedures from fresh tumor tissues and from cultured melanoma cells and their spent culture media derived from the same melanoma patients. This includes removal of HLA antigens and purification and physicochemical characterization of MAA which are defined biologically by their ability to elicit specific delayed cutaneous hypersensitivity reactions in melanoma patients.

Progress: Human melanoma-associated antigens (MAA), when solubilized from cultured tumor cells by extraction with 3M KCl, were shown to be antigenic and elicited a cell-mediated immune reaction as determined by delayed cutaneous hypersensitivity responses (DCHR). Between 75-80% of melanoma patients showed positive DCHR, while only 25% of patients with other neoplasms reacted. Antigen purification was achieved by KBr gradient ultracentrifugation, cellulose (DEAE), ion-exchange chromatography, and preparative isoelectric focusing (IEF) on Pevikon C870. A new method was developed to remove histocompatibility antigens as well as lipids and lipoproteins from MAA utilizing KBr gradient ultracentrifugation. Several *in vitro* antibody-binding assays developed in our laboratory were utilized to assess their potential to quickly evaluate the progress of antigen purification.

Significance to Cancer Research: The continuous availability of chemically and biologically defined purified human melanoma-associated antigens should prove advantageous to establish their utility to produce specific xenoantibody and to test the efficacy of these reagents for tumor diagnosis, therapy, and in monitoring response to therapy.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $160,931

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator: Dr. Argyrios N. Theofilopoulos
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: NO1-CB-53952
Starting Date: 5/16/75 Expiration Date: 5/15/77

Goal: Detection and quantitation of immune complexes (ICs) in sera of patients with malignancies; determining prognostic significance of ICs in monitoring these patients; identification and possible isolation of the tumor antigens in ICs; and production of antisera against tumor antigens in ICs.

Approach: Levels of ICs will be determined by the Raji cell radioimmunoassay. ICs will be isolated using Raji cells or insolubilized conglutinin as substrates. Isolated antigens or ICs will be used for the production of antisera in experimental animals.

Progress: A sensitive and simple procedure for the detection and quantitation of soluble complement-fixing IC in human sera has been developed by utilizing complement receptors on Raji cells. Over 500 patients with various malignancies were studied using this assay. Abnormal levels of ICs were found in from 16 to 52% of patients with different types of tumors. The highest incidence of elevated IC was found in colon carcinoma, melanoma, osteogenic sarcoma, breast carcinoma, and carcinoma of the stomach. The incidence of positivity and the amount of IC found increased with increasing tumor size and with metastatic spread. Immunofluorescence and isotope-labeled antibody techniques showed that IC from sera of patients with melanoma, osteosarcoma, and colon carcinoma which bound to the complement receptors on Raji cells contained respective tumor cell antigens. Techniques were developed for concentrating and isolating preformed IC from human serum as well as sera of experimental animals with serum sickness and transplantable tumors by binding to Raji cells or to insolubilized bovine conglutinin.

Significance to Cancer Research: Tumor antigen-antibody complexes have been implicated as modulators of tumor immunity. Development of techniques for their identification and quantitation may help in monitoring the course of the disease and the effect of treatment in these patients. Isolation of tumor antigens and production of antisera against them will be of extreme importance in understanding the pathogenesis of malignancies and in developing specific diagnostic procedures.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $85,569

CONTRACT RESEARCH SUMMARY

Title: Improvement of Methods for Isolation and Preservation of Lymphocytes

Principal Investigator: Dr. Stuart Schlossman
Name/Addressss Sidney Farber Cancer Institute
Performing Organization: Boston, MA

Contract Number: NO1-CB-43885
Starting Date: 5/16/74 Expiration Date: 5/15/77

Goal: To define techniques for the isolation of individual subsets of human lymphocytes which are viable and functionally intact, and to attempt cryopreservation of such lymphocyte subsets with the view of recovery and preservation of their functional properties.

Approach: The subsets of lymphocytes were assayed for structural integrity and viability, and analyzed in vitro by functional assays. Cell surface properties were defined before and after cryopreservation by three markers which have been particularly useful in this laboratory and included FITC-Fab anti-F(ab), aggregated gammaglobulin for Fc receptors, and anti-Ia-antisera.

Progress: The contractor concentrated in the past year on analyzing functional properties of lymphocytes and the effect of varying conditions in freezing and thawing on the recovery of subsets of lymphocytes, particularly the Thl^+ subset of human T cells capable of responding in MLC and the Thl^- subset of lymphocytes which express cytotoxic activity. Attempts to change conditions which lead to a diminished viability of the recovered cell population severely affect ADCC activity. In addition, Fc binding was analyzed, concentration of Ia antigen and surface immunoglobulins on 40 individuals with CLL and following freezing and thawing. No changes in the surface properties of the cells were obtained by storage upwards of one year, and conditions affecting viability and recovery had little or no effect on the cell surface properties of the cells. Diminished total viability could be circumvented by recovery of cells following Ficoll-Hypaque centrifugation. No specific enrichment or deletions of effector functions were seen following cryopreservation.

Significance to Cancer Research: The cryopreservation of lymphocytes is important since it permits the recovery of populations of cells of defined structural and functional integrity for studies of immune responsiveness of cancer patients as well as to allow exchange among laboratories of defined populations of cells.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $44,649 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Stuart Schlossman
Name/Address
Performing Organization: Sidney Farber Cancer Institute
Boston, MA

Contract Number: NO1-CB-43964
Starting Date: 5/16/74 Expiration Date: 5/15/77

Goal: Development of procedures for more effective and reproducible fractionation of mononuclear cells from human peripheral blood.

Approach: Peripheral blood from normal individuals and from patients with carcinomas will be studied. The following cell types will be isolated in suspension with high yields, purity, and viability: T lymphocytes, B lymphocytes, and monocytes. The isolated cells will be characterized for viability, morphology, retention of membrane markers, and retention of immunological functions.

Progress: The contractor has focused on identification of surface membrane determinants on human lymphocyte subpopulations analogous to TL, Ly, theta, and Ia determinants present on murine lymphocyte subpopulations. The analysis of surface determinants has aided work on the development of new techniques for the isolation of highly purified subclasses of human lymphocytes and permitted development of new in vitro techniques to discriminate the functional behavior of the isolated subclasses. Studies have also been done on human B cells, specifically analysis of the signals required for B-cell differentiation and control of that differentiation by T cells. Progress has been made on analysis of the role of human Ia-like determinants on the surface of lymphocytes and monocytes in the generation of cell-mediated immune responses.

Significance to Cancer Research: NCP Objective 5 Approach 1

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $81,663

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Dr. Jorgen E. Fogh
Name/Address
Performing Organization: Sloan-Kettering Institute for Cancer Research
New York, NY

Contract Number: NO1-CB-43854
Starting Date: 8/8/73 Expiration Date: 8/7/77

Goal: To further develop procedures for encouraging tumor cell growth in culture and to perform tests to determine if cells in culture are indeed neoplastic rather than normal cells. To make cultures available to other investigators.

Approach: Human tumor cell lines are established in culture from surgical specimens and are collected from other investigators. They are characterized in this laboratory as to malignancy, cell type, and special features as needed for individual investigations.

Progress: Three hundred ninety established cell lines, derived from human tumors, have been collected from cultures. The experience gained during the past period permits cell line establishment from certain types of tumors much more frequently than in the past. Methods have been employed to assess malignant characteristics of the cultured cells, consistency in terms of tumor cell type with the tumor of origin, and special features of the individual cell lines. These methods have provided information on cell morphology, nutritional requirements, growth in vitro, tumor production in the nude mouse, chromosomal characteristics, poliovirus susceptibility, and isozyme patterns. Only a small proportion of the collected lines are suspected of HeLa cell contamination. Mycoplasmas and other contaminants of a number of the lines have been eliminated from all but 13 lines, still mycoplasma contaminated. The major part of the lines, and many early cultures, are frozen in liquid nitrogen and, when sufficiently characterized, available for distribution to collaborating investigators. A computerized catalog of clinical background information and cell characterization data is being constantly updated. A computerized data file of all cell specimens stored in liquid nitrogen has been established during the last year. One thousand seven hundred forty-four cultures of a variety of human tumor cell lines were shipped to investigators in the United States and abroad. Efforts are made to obtain data derived from their studies.

Significance to Cancer Research: The collection of well-characterized contamination-free human tumor cell lines is pertinent for many disciplines of experimental and clinical cancer research.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 10/31/73
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $224,000 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-Associated Antigens

Principal Investigator: Dr. Kenneth O. Lloyd
Name/Address Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, NY

Contract Number: NO1-CB-64021
Starting Date: 6/30/76 Expiration Date: 6/29/77

Goal: The aim of this project is to purify human ovarian cancer antigens which can be detected using heterologous antisera and to develop sensitive assays for these antigens. These assay systems will then be used to detect the antigens in sera of women with ovarian cancers and of suitable controls to determine their usefulness in the diagnosis of ovarian cancer.

Approach: The approach entails the use of heteroimmune sera to detect and assay tumor-associated antigens in ovarian tumors and sera from cancer patients.

Progress: The antigenicity of ovarian tumors is well documented in the literature; numerous antigenic reactions have been described but the corresponding antigens have not been well characterized. Five ovarian tumor-associated antigens were identified using heteroimmune sera. Two of these appear to be new ovarian antigens with potential usefulness in immunodiagnosis. The others are previously described antigens: pregnancy-zone protein (PZP), carcinoembryonic antigen (CEA), and normal glycoprotein (NGP). Purification of the two new antigens (OvC-1 and OvC-2) is in progress. OvC-1 and OvC-2 have molecular weights of about 60,000 and 45,000, respectively. OvC-2 is confined to ovarian tumors, whereas OvC-1 has a somewhat more widespread distribution, being also found in some other tumors and in small amounts in some normal tissues (lung and kidney). Once the antigens have been purified, they will be used to raise more specific antisera and an assay system will be developed for their detection in sera of cancer patients and controls.

Significance to Cancer Research: Ovarian cancer has a high death rate because diagnosis and surgical removal are often critically delayed due to its deep location and lack of early symptoms. Immunodiagnostic assays are aimed at the early detection of ovarian tumors in asymptomatic women.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Role of Antibody-Dependent Cell-Mediated Cytotoxicity in Tumor Immunity

Principal Investigator: Dr. Osias Stutman
Name/Address
Performing Organization: Sloan-Kettering Institute for Cancer Research
New York, NY

Contract Number: NO1-CB-53894
Starting Date: 5/1/75 Expiration Date: 4/30/77

Goal: Determine the importance of cytotoxicity of syngeneic tumor cells by antibody-dependent cell-mediated mechanisms in host resistance to tumor growth or spread, and whether the presence of such antibodies would be useful for the detection of malignant or premalignant lesions and for monitoring tumor growth.

Approach: Study the role of antibody-dependent cell-mediated cytotoxicity in tumor immunity in a mouse syngeneic system using spontaneous mammary adenocarcinomas.

Progress: A reliable assay for antibody-dependent cell cytotoxicity (ADCC) using a syngeneic mouse system was developed. The antibody has activity in IgG subclasses and not in IgA or IgM and it is thymus dependent in development. The antibody is produced by hyperimmunization with mammary tumor cells or derived from tumor-bearing C3H mice. Adherent C3H mammary tumors prelabeled with ^{3}H-proline are target cells. Effector cells are from normal C3H or C3Hf donors. Effector cells are relatively radioresistant (especially those derived from blood or peritoneal cavity; those from spleen are less sensitive); treatment of the donors with ^{89}Sr does not affect the spleen cell effectors; concerning surface markers, the effector cell is negative for all T markers while B-specific markers reduce their effectivity. The in vitro responses can be inhibited by relevant free antigen, excess free specific antibody, excess free irrelevant soluble Ig, aggregated or insoluble Ig, and relevant or irrelevant antigen-antibody complexes.

Significance to Cancer Research: (NCP Objective 6 Approach 4 and Objective 3 Approach 4)
Studies of cells involved in the immune destruction tumors are an important part of Baseline Information Flow in the Tumor Immunology Program.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $66,744

CONTRACT RESEARCH SUMMARY

Title: Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins

Principal Investigator: Dr. Om Bahl
Name/Address State University of New York
Performing Organization: Buffalo, NY

Contract Number: NO1-CB-43989
Starting Date: 5/1/74 Expiration Date: 7/30/77

Goal: Isolation and characterization of endoglycosidases which are capable of degrading the polysaccharide moiety of glycoproteins.

Approach: The general experimental approach involves (1) the screening of various animal, plant, and microbial sources for endoglycosidase activity, and (2) the enrichment of endoglycosidase activity in the culture medium of bacteria grown on specific carbon sources. A correlated aspect of the work is to prepare suitable substrates for endoglycosidases either from the naturally occurring glycoproteins or by chemical synthesis.

Progress: Glycoproteins have assumed a great deal of importance because of their involvement in a number of disease states, particularly alterations in cell surface glycoproteins during malignancy. Availability of the endoenzymes will not only facilitate understanding of the molecular structure of glycoproteins but also help in delineating the specific parts of the structure related to the disease state. Initial efforts were directed toward the preparation of suitable substrates for the enzymes. Two types of substrates were prepared, one ^{3}H-labeled obtained by the controlled periodate oxidation and reduction and second by the reduction of the carboxyl groups of sialic acid in the glycopeptide from α_1-acid glycoprotein. With the help of these substrates several sources have been screened for endoglycosidase activity. Results of these studies have not been very promising. Consequently, attention was focused on the elaboration of the enzymes by the enrichment technique, i.e., isolation of bacteria which can grow on specific carbon sources, such as polysaccharides, galactomannans, chitin, and yeast mannan. Several bacteria have been thus isolated that produce endoglycosidases which are released in the culture media. Work is in progress to isolate and characterize these enzymes.

Significance to Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $45,103 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Human Tumor-Associated Antigens

Principal Investigator: Dr. Ilkka P. T. Hakkinen
Name/Address Turku University
Performing Organization: Turku, Finland

Contract Number: NO1-CB-64070
Starting Date: 6/30/76 Expiration Date: 6/29/77

Goal: The project aims to establish the FSA test in screening for early gastric cancer, including characterization of the FS-antigen, standardization of the reaction and testing the method by screening an unselected population for gastric cancer as well as discriminating between benign and malignant gastric ulceration, using a clinical series.

Approach: Acid glycoproteins from gastric juice are tested by immunodiffusion against fetal sulphoglycoprotein antiserum. Preparation of the antiserum will be standardized. Subjects secreting FSA will be submitted to clinical examination (gastroscopy). The entire cohort will be followed for two years and the cancer rates of an extensive series compared with existing statistics.

Progress: More than 95% of gastric cancers secrete FSA in gastric juice. The number of people over 40 who secrete FSA does not exceed 7%. It can be seen that clinical examination of this high-risk group is within the limits of feasibility. Further studies on the structure of FSA and on the development of FSA-RIA are in progress. About 30,000 people have been tested by the FSA method. Clinical findings support the working hypothesis that FSA is a marker of a high-risk group for gastric cancer and that early cancers can be detected by this method. Up to now, 21 out of 24 gastric cancers in the FSA-positive material were operable and without metastasis which gives promise of a better prognosis.

Significance to Cancer Research: If the method can be proved to be practical, it will provide a new tool for the early diagnosis of gastric cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator: Dr. Ralph E. Schrohenloher
Name/Address University of Alabama
Performing Organization: Birmingham, AL

Contract Number: NO1-CB-53893
Starting Date: 5/1/75 Expiration Date: 4/30/77

Goal: Establish the presence of circulating immune complexes in cancer and determine the identity, levels, and diagnostic or prognostic value of these materials.

Approach: Develop sensitive and quantitative methods for soluble immune complexes where the antigen is not known, systematically screen sera from a broad cross-section of cancer patients to establish the existence of circulating immune complexes, and establish the identity of the complexes by physicochemical and immunochemical techniques.

Progress: A radioassay based on reaction with human monoclonal rheumatoid factor has been developed. Its capacity to detect circulating immune complexes containing IgG was established using sera and other body fluids from patients with connective tissue diseases associated with a high incidence of these materials. Characterization of the detected materials indicated that the assay is capable of revealing small complexes sedimenting intermediate to 7S and 19S in addition to larger complexes sedimenting at 19S and faster. A radioassay for immune complexes based on inhibition of the binding of radiolabeled C1q to latex-aggregated IgG particles was also developed. The method is rapid, sensitive and semi-quantitative, but lacks the specificity necessary for detection of immune complexes in cancer sera. For this reason a modified assay using C1q is under development. Application of the monoclonal rheumatoid factor assay to screening sera from cancer patients has been initiated.

Significance to Cancer Research: Studies on the occurrence of immune complexes in cancer are of potential significance in understanding the immunobiology of the disease. Procedures for immune complexes also show promise as aides in monitoring disease activity.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $92,741

CONTRACT RESEARCH SUMMARY

Title: Development of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. Ronald Stevens
Name/Address University of California
Performing Organization: Los Angeles, CA

Contract Number: NO1-CB-43860
Starting Date: 9/1/73 Expiration Date: 12/31/76

Goal: To identify new tumor-associated antigens in carcinomas of the colon, stomach, lung, breast, prostate, and bladder which might be useful for immunodiagnosis.

Approach: Extract antigens from these carcinomas and from comparable normal tissues and from fetal tissues and prepare heterologous antisera against each of the antigens. Antigens shall be purified and antisera rendered specific for tumor-associated antigens. Radioimmunoassays shall be developed for the detection of each antigen in serum or urine.

Progress: Four antigens have been detected and may represent lung tumor-associated antigen. An antigen(s) NL-1 has been identified and is characteristic of normal lung and of some adenocarcinomas of the lung. Another antigen of M.W. 35,000 has been detected and seems to be confined to and produced by lung tumor cells. Two other antigens were found to be present in extracts of lung tumor tissue and absent in extracts of normal lung tissues. Preliminary radioimmune assays have been developed.

Significance to Cancer Research: New and more specific immunodiagnostic tests for the common types of human cancer are needed.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 7/23/75
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Monocytes

Principal Investigator: Dr. David Talmage
Name/Address University of Colorado Medical Center
Performing Organization: Webb-Waring Lung Institute
Denver, CO

Contract Number: NO1-CB-53900
Starting Date: 5/16/75 Expiration Date: 5/15/77

Goal: To isolate monocytes from human peripheral blood, both from normal donors and from donors having carcinomata, and to test these cells for functional activity and markers.

Approach: Mononuclear cells are being separated from whole blood; monocytes are then isolated from the lymphocytes by counterflow centrifugation using a specially designed chamber.

Progress: Highly pure populations of monocytes, and also of lymphocytes, are being obtained by the described approach. Purity now averages about 95%. It should be noted, however, that it is not possible to separate all monocytes from all lymphocytes, a mixed population being obtained between the pure fractions. Normal monocytes defined by these assays have a bimodal volume spectrum, preventing their separation from the lymphocytes. In addition to phagocytic ability and peroxidase activity, the isolated monocyte populations are being tested for their role in the stimulation of lymphocytes by Con A. In normal donors, monocytes are an absolute necessity for stimulation when the culture includes homologous plasma. Furthermore, the degree of stimulation appears to be a linear function of the percentage of monocytes present at least up to a monocyte-lymphocyte ratio of 40%. Even at this level, however, stimulation is much less marked than for cultures which use FCS instead of homologous serum. In studies with cancer patients, it was found that phagocytosis is often, though not always, impaired, that peroxidase activity is very variable, and that cell volume spectrum is usually bizarre.

Significance to Cancer Research: The monocyte is known to play an important role in the immune system through its ability to stimulate lymphocytes. Dysfunction of the immune response in cancer can therefore be examined by comparison of experiments using monocytes isolated from normal donors and cancer patients.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $51,528

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Circulating Tumor-Associated Antigens for Diagnosis of Cancer

Principal Investigator: Dr. James W. Osborne
Name/Address University of Iowa
Performing Organization: Iowa City, IA

Contract Number: NO1-CB-43891
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: Perform studies of circulating tumor-associated antigens in animal carcinomas that would serve as a model for common human cancers and that are potentially useful in developing immunodiagnostic tests for cancer.

Approach: Study antigens associated with radiation-induced intestinal tumors in rats. Demonstrate and isolate circulating tumor-associated antigens and quantitate the antigen. Study, if possible, the relationship of antigen appearance to premalignant lesions and determine the levels of antigens in relation to tumor size.

Progress: A tumor-associated protein (TAP) was found in an x-irradiation-induced intestinal adenocarcinoma of the rat. The glycoprotein was associated with the tumor cell membranes and shared common antigenic determinants both with rat fetal protein and perchloric acid-soluble protein isolated from the serum of tumor-bearers. Some tumor-bearing animals had decreased levels of the circulating TAP, but a definite correlation between tumor size and serum levels of TAP has not yet been achieved. The immunological activity of the TAP was acid labile, yet alkali and heat stable. Endo- and exopeptidases eliminated discernible immunological activity, but nucleotidases and neuraminidase did not. Purification of 3M KCl extracts was done. A radioimmunoassay for the purified protein was recently developed, but further purification is needed to increase the specific activity of the radiolabeled derivative so that serum levels may be determined.

Even though the oncofetal protein has not yet been fully characterized, it has been determined that KCl extracts block the cytotoxicity of lymphocytes (from tumor-bearing rats) to cultured cells of the tumor. This indicated that the tumor is immunogenic in the host and that blocking factors are also present.

Significance to Cancer Research: The study of this animal model of intestinal carcinoma is of potential importance for determining whether the level of circulating oncofetal protein will be a useful immunodiagnostic tool for cancer. It is especially significant since the morphological and immunological features of the tumor are quite similar to colon and rectal cancer in man.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-Associated Antigen

Principal Investigator: Dr. Robert Humphreys
Name/Address University of Massachusetts Medical School
Performing Organization: Worcester, MA

Contract Number: NO1-CB-64072
Starting Date: 6/30/76 Expiration Date: 6/29/77

Goal: The objective of this study is to identify and isolate membrane proteins from leukemic cells in order to raise rabbit antibodies which may be used to diagnose biologic subsets of leukemias and lymphomas by cytotoxic or immunofluorescent assays.

Approach: In order to identify subsets of leukemias, membrane proteins from leukemic cell lines are examined by [^{35}S]methionine, autoradiographic techniques in SDS polyacrylamide slab gel electrophoresis. T- and B-specific proteins are identified. These proteins are then isolated by traditional protein chemical techniques. Antisera to these proteins are assayed for their specificity for leukemia or for subsets of leukemias.

Progress: Prior to this contract, the investigator found and isolated p23,30, a B lymphocyte-specific marker which identified a biological subset of patients with acute lymphoblastic leukemia. In the first year of this contract the focus has been on comparing SDS polyacrylamide membrane patterns of T and B lymphocytic leukemia cells. B cell patterns are similar to each other while two cell lines, HSB and CEM, differ from each other and from B-cell patterns. Putative B- and T-specific membrane proteins have been identified in these analytic gels. Several proteins have been isolated from membranes by traditional techniques. One of these proteins, p46 from the CEM line, appears to be specific for a subpopulation of normal peripheral blood T lymphocytes. Antisera to other proteins are cytotoxic for lymphocytes and are being characterized. A B-cell antigen of 92,000 daltons has been found. In the coming year these antigens will be characterized in detail and the use of antisera to them for leukemia diagnosis will be tested.

Significance to Cancer Research: The identification of biological subsets of leukemias, as in the case of p23,30-positive ALL patients, permits correlation to the clinical course. These assays may in time affect the selection of chemotherapeutic protocols based on the identification of biological subsets of cancers, or the antisera may be used to modify the immune response in patients with cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Dr. John S. Najarian
Name/Address University of Minnesota
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-33915
Starting Date: 6/30/73 Expiration Date: 4/30/78

Goal: Serum is collected from individuals in the high cancer risk age group (50 to 70 years of age) during their annual visit to a cancer detection center. The serum is coded, frozen, and stored for future evaluation of potentially promising immunodiagnostic tests for cancer.

Approach: In addition to collection of the serum samples, detailed history is kept which includes the date cancer is detected, and the date and cause of deaths of any members in the study. When the study and collection are completed, this information will be used to designate at least three groups of serum samples: those who never develop cancer; those who develop cancer within a year of the sample; and those developing cancer before the sample is taken. when a new immunodiagnostic test of promise is developed, its potential in early detection of cancer can be validated very quickly by means of this serum bank.

Progress: Since the study started, a total of 5,219 asymptomatic adults have been examined and serum collected for the study. Follow-up on this group indicates that 2,018 have returned for the second visit, and 1,001 for the third visit. Total exams on this population performed have been 8,565. To date, there have been 35 deaths in the population and a total of 94 malignancies, 57 of these were detected at the time of the examination, and 37 developed between visits. Documentation of the malignancy includes the type of tumor, its location, date detected, date confirmed, and the type of treatment the patient received.

Significance to Cancer Research: The importance of early diagnosis of cancer has been repeatedly demonstrated and can result in increasing the life span of those involved. The collection of a bank of serum from individuals whose cancer histories have been carefully and accurately documented can reduce the time required to evaluate the potential of a new diagnostic test from many years to a few days. This results in tremendous savings of money and, more importantly, reduces the time required for initial determinations of value and establishments of efficacy.

Project Officer: Mrs. Judith M. Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $80,096

CONTRACT RESEARCH SUMMARY

Title: Immunofluorescent Detection of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Dr. George J. Friou
Name/Address
Performing Organization: University of Southern California
Los Angeles, CA

Contract Number: NO1-CB-43881
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: Study of sera of patients with breast carcinoma for presence of antibodies to autologous tumor cells using immunofluorescence, studying surface and intracellular antigens, immunoglobulin class and specificity of observed reactions, and relationship of antibodies to stage and course of disease, screening tests for cell-mediated immunity, and presence of autoantibodies.

Approach: Primary site specimens were obtained from untreated patients. Preoperative, postoperative, and follow-up sera, clinical, pathological data collected. Preoperative sera were tested against autologous viable tumor cells and fixed cell smears, and selected follow-up sera similarly tested. Ig class of antibodies in selected sera was determined. Allogeneic tests and absorptions have been used to evaluate specificity of observed reactions.

Progress: Initial studies included lung and colon as well as breast, but later work was restricted to breast carcinoma patients. Studies prior to the present contract year established that antibodies to autologous surface and intracellular antigens were present in 16/41 (39%) and 29/52 (55%) of breast cancer sera, respectively. Allogeneic tests with cancer sera showed consistency of reactions; controls showed no surface reactions, and 12-13% intracellular reactions. Sera of patients with biopsy-confirmed fibrocystic disease and fibroadenoma showed reaction frequencies with intracellular antigens similar to sera of cancer patients.

Current year studies have included absorptions showing shared antigens between ductal carcinoma and fibroadenoma. Stage 4 sera fail to react in autologous reaction, but cross-testing of stage 1, 2 and 4 sera indicates this to result from antigenic change in stage 4 cells rather than absence of antibody in stage 4 sera. Other clinical correlations, autoantibody studies, and relationship to embryonic antigens are in completion phase.

Significance to Cancer Research: Antibodies detected may assist in cancer diagnosis, or in screening for premalignant lesions. Failure of reactions of positive sera with stage 4 cells has important conceptual relevance to understanding of mechanisms of tumor progression.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer of Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: New In Vitro Techniques to Evaluate Cell-Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. John T. Harrington
Name/Address
Performing Organization: University of Texas Medical School
San Antonio, TX

Contract Number: NO1-CB-43893
Starting Date: 6/1/74 Expiration Date: 10/31/77

Goal: To establish methods for measuring the cell-mediated immune response of tumor patients to their tumor antigens.

Approach: To develop and apply the agarose droplet micromethod for measuring cell migration inhibition -- an in vitro correlate of cell-mediated immunity -- in patients with bladder, lung, and breast carcinoma using antigens derived from autologous tumor material and from established tumor cell lines.

Progress: This research is being conducted in three overlapping phases: (1) Development of the agarose droplet method using a murine tumor allograft rejection model. These studies, which have been completed, permitted rapid technical development of the method and verification of its use for testing immunity to cell surface antigens. Cell freezing techniques, antigen extraction methods, and definition of the cell-mediated response in this model were also accomplished. (2) Development of a human agarose droplet micromethod. This work, which has been completed, tested the migration inhibition response of human tuberculin-sensitive individuals to PPD. Human assay conditions have been verified and optimal migrating indicator cells chosen. (3) Application of the micromethod to assess human tumor immunity. These studies are now in progress and have demonstrated positive migration inhibition reactions in bladder, lung, and breast carcinoma patients to extracts of tumor extracts of cultured cell lines derived from these tumors. Further definition of the specificity, reproducibility, and clinical correlation of these reactions are in progress.

Significance to Cancer Research: The accurate assessment of antitumor immunity in cancer patients may be important to both diagnostic and treatment decisions. Development of appropriate methods is critical to this effort.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $81,842

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Human Tumor-Associated Antigens

Principal Investigator: Dr. E. Russell Alexander
Name/Address University of Washington
Performing Organization: Seattle, WA

Contract Number: NO1-CB-64068
Starting Date: 6/1/76 Expiration Date: 5/31/77

Goal: To develop and purify an antigen and antiserum which are associated with epidermoid carcinoma of the cervix. To develop methods to use that antigen and antiserum in serological studies.

Approach: Monospecific rabbit antiserum is being prepared against the tumor-associated antigen. Affinity chromatography is being used for purification of antigen based on monospecific antiserum, which purified antigen and monospecific antiserum will be used to detect circulating antibody and antigen by different serologic techniques.

Progress: Work has progressed in the development of a pure monospecific antiserum. When this serum reacts against crude tumor antigen in two-dimensional immunoelectrophoresis, a single precipitin line results. This antigen has been demonstrated by two-dimensional immunoelectrophoresis in 19 cervical cancer tissues, but was absent in 10 other gynecologic cancers and 9 normal gynecologic tissues (including 7 specimens of normal cervical tissue). The antigen is a protein with a molecular weight of approximately 74,000 daltons. When 7 cervical cancer specimens were examined by immunofluorescent antibody methods using "monospecific" antiserum, antigen was seen in both membrane and cytoplasm of cervical cancer cells and was absent in 5 specimens from other gynecologic tumors and 4 normal cervical and 2 normal ovarian preparations. By rocket electrophoresis, the antibody did not detect circulating antigen in serum of cancer patients. However, this antibody did detect circulatory antigen by Ouchterlony immunodiffusion in 2/30 cancer patients' sera and 0/30 control sera.

Significance to Cancer Research: The basis of this study is to develop an immunodiagnostic method for early detection of cervical cancer, and to determine the relationship of such a detection to the development of abnormal cervical cytology (Papanicolaou smear). Of particular concern will be study of the origin and character of this antigen and any possible relationship to the cause of cervical cancer.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Hans Wigzell
Name/Address Uppsala University
Performing Organization: Uppsala, Sweden

Contract Number: NO1-CB-43883
Starting Date: 6/25/74 Expiration Date: 6/24/77

Goal: Human peripheral blood mononuclear cells constitute a population of cells, morphologically in a superficial way looking alike but with highly distinct immune functions. To separate and define such populations constitutes our major goal.

Approach: Development of surface markers, preferentially of a unique character signifying a given subgroup of cells, is the most ultimate approach allowing rapid fractionation and purification of blood mononuclear cells. A battery of fractionation procedures linked up with functional tests was applied in order to analyze this question.

Progress: It has been possible to develop a most sensitive and quantitative technique for the detection of membrane-associated IgG or antigen-molecules using iodinated protein A. Using this technique subsequently the Fc receptor distribution of subgroups of human blood mononuclear cells was analyzed, as well as HLA, and beta 2-microglobulin distribution pattern. Whereas striking differences exist in Fc distribution patterns, HLA and beta 2-microglobulin distribution profiles are very similar between subgroups. Protein A has also been found to be a human B-cell mitogen, a useful marker for blood B cells in man. New techniques have been developed as to allow simple fractionation of C3-receptor-bearing cells using C3-coated zymosan-particles. Helix pomatia A agglutinin can be shown to serve as a useful marker in the selective separation of T lymphocytes as well as natural killer cells. Specific analysis of separated subpopulations of human mononuclear cells labeled as to surface glycoproteins using sodium borohydride techniques indicates the existence of distinct subtype-specific glycoproteins. Antisera against such "bands" are being induced.

Significance to Cancer Research: Human peripheral blood constitutes a major, simple source of human mononuclear cells. Understanding this complex population as to surface markers as well as functional capacity is directly related to understanding how the immune system in that individual may function.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Antibodies to Human Organ- or Tissue-Associated Antigens

Principal Investigator: Dr. Jen-Fu Chiu
Name/Address Vanderbilt University
Performing Organization: Nashville, TN

Contract Number: NO1-CB-53896
Starting Date: 6/16/75 Expiration Date: 6/15/77

Goal: To detect antibodies to human organ- or tissue-associated antigens to use in study of antigenic profile of normal and neoplastic cells, both from biopsy and tissue culture.

Approach: Obtain specific antibodies to chromosomal nonhistone proteins associated with homologous DNA, and measure in tumor cells the quantitative decrease or loss of normal antigens and increase of tumor-specific antigens. Distribution and expression of these antigens will be studied in relation to disease type, stage of disease, and prognosis.

Progress: Tissue-specific antisera were obtained against human lung carcinoma, breast carcinoma, and HeLa S_3 cell dehistonized chromatin. The specificity of these antisera was determined by complement fixation. Only chromatins from specific tissues or cells fixed the complement significantly, while chromatins isolated from other human tumors were negative. Localization specificity of antisera against human lung carcinoma was studied with lung carcinoma and normal lung adjacent to tumor. The localization studies indicated that there is no tumor-specific antigen present in normal lung. Studies on the immunospecificity of chromatins isolated from G_1, S, and G_2 phases of HeLa S_3 cell cycle showed that chromatin isolated from G_2 showed much higher immunoactivity than chromatin isolated from other phases. Human lung carcinoma chromatin was fractionated to study the immunoactivity of its components. DNA, RNA, and histones were not responsible for the observed immunospecificity. Two subfractions of nonhistone proteins represented most of chromosomal nonhistone proteins.

Significance to Cancer Research: Developing specific and sensitive antibodies against human organ- or tissue-associated antigens, including tumors, for immunodiagnosis, has a high priority.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 4/25/75
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development of Macrophage Electrophoretic Mobility Assay for Malignant Disease

Principal Investigator: Dr. John A. Pritchard
Name/Address Velindre Hospital
Performing Organization: Whitchurch, Cardiff, Wales

Contract Number: NO1-CB-53956
Starting Date: 6/29/75 Expiration Date: 9/29/77

Goal: The use of a biochemical technique to isolate and identify an effector compound released by cancer-sensitized lymphocytes, or by stimulation with appropriate antigen; development of a refined apparatus for the measurement of macrophage mobility in the bioassay for the presence of the effector compound. Where possible, both systems to be appraised by a series of coded blood samples to establish the clinical value of the macrophage electrophoretic mobility (MEM) test for malignant disease.

Approach: Large-scale preparation of the effector compound, macrophage slowing factor (MSF), to be undertaken. The active fractions to be pooled (by ultrafiltration) and used to raise a rabbit anti-MSF serum. Assessment of the rank Mk.III electrophoresis system for the simple measurement of macrophage mobilities.

Progress: The mediator of macrophage slowing (MSF) has been found to have a molecular weight of 20-23,000 and this is consistent with published data. It is stable at 56°C and a storage (4 weeks) at -10°C. Bioassay, using macrophage mobility reduction, showed that activity was retained at 10^{-3} dilution. Conventional means of biochemical analysis could not establish its presence. Repeated attempts using up to 50 individual lymphocyte preparations to produce MSF, which was subsequently pooled and ultrafiltered, were unsuccessful in raising an antisera. The rank Mk.III electrophoresis system has been developed for ease of operation in the measurement of all mobilities and has been assessed in the operation of the MEM assay and found to produce similar results as obtained with the Zeiss cytopherometer with considerably less operator fatigue. Results on a coded series of 240 samples are in the process of analysis.

Significance to Cancer Research: The MEM test has been proposed as a sensitive laboratory test for malignant disease. This can only be truly assessed in the routine operation of the test in conjunction with accepted clinical procedures.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $6,618 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Detection of Circulating Antigen-Antibody Complexes in Cancer

Principal Investigator: Dr. Dieter Burger
Name/Address Washington State University
Performing Organization: Pullman, WA

Contract Number: NO1-CB-63990
Starting Date: 9/1/75 Expiration Date: 8/31/77

Goal: To isolate immune complexes from the circulation and identify the role which these antigen-antibody complexes may play during enhancement or regression of tumor growth.

Approach: Affinity chromatography with immobilized equine complement Clq is used as a sorbent for isolation of complement Clq-fixing immune complexes using feline leukemia as a model for human cancer. Purified immune complexes are quantitated and then identified. These data will be correlated with the clinical status of the animal, presence of leukemia group-specific antigen (gs-antigen), and the titer or antitumor antibody.

Progress: During the present contract year circulating immune complexes were isolated frequently from cats without exposure to feline leukemia virus. A detailed analysis by virus neutralization and fluorescent antibody tests demonstrated that the antibody specificity of these complexes was directed against feline caliciviruses, the causative agents of a common feline respiratory infection. During a chronologic study of circulating immune complexes in two calicivirus-infected kittens, it was observed that the virus infection persisted in the respiratory tract during the entire observation period, whereas the complexemia was temporary (3 weeks) and declined to negative values during the observation period of five weeks. Immune complexes isolated from eight cats which carried protective anti-leukemia (FOCMA) antibody had higher IgG concentration (X 208 ug/ml) than four sera from leukemic, FOCMA negative cats (94 ug/ml). In one cat with a rapidly progressing tumor no complement-fixing immune complexes were found.

Significance to Cancer Research: Our affinity chromatography technique can be used to isolate circulating immune complexes from kryptic disease processes such as cancer, and it may aid in identifying the causes of enhancement of tumor growth.

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $56,528

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor-Associated Antigens in Carcinoma of the Lung

Principal Investigator: Dr. Robert W. Veltri
Name/Address
Performing Organization: West Virginia University
Morgantown, WV

Contract Number: NO1-CB-43890
Starting Date: 5/16/74 Expiration Date: 5/15/77

Goal: Search for new tumor-associated antigens in carcinoma of the lung, which may be more useful in immunodiagnosis than currently available markers.

Approach: Lung tumor tissue, normal lungs, and embryonic lungs are processed by a variety of chemical, physical, and mechanical procedures. Antisera to these soluble and membrane extracts are raised in rabbits and absorbed until reactive only with tumor extracts. The antigens thus identified are further characterized and purified for the development of sensitive serum screening procedures.

Progress: With the above approach three proteins have been identified in soluble extracts of human lung carcinomas. These have been called LTAA-1, 2,3. LTAA-1 has been identified as a serum protein present in Cohn fraction IV-4 of human serum and present in lung tumors in large excess. LTAA-2 has been identified as a ferritin and LTAA-3 has been shown to react with commercial antiserum to human lactoferrin. These three antigens have been purified, large volumes of monospecific antiserum prepared, and RIAs are being developed to screen for one or more of them in sera of patients with lung cancer, benign lung disease, and in normal controls. Two other proteins, LTAA-4,5, have been identified in Triton X-100 extracts of lung tumor membranes. As was the case for soluble LTAA-1,2,3 the membrane-associated LTAA-4 and 5 are not CEA, AFP or β_2-microglobulin. LTAA-4 and 5 have been separated by ion exchange chromatography and monospecific antisera are being raised to these antigens. Again the antisera will be used in the development of diagnostic immunoassays. Major emphasis is being directed toward development of RIAs for lung TAAs 3, 4 and 5.

Significance to Cancer Research: (NCP Objective 5 Approach 4)

Project Officer: J. Frederic Mushinski, M.D.
Program: Tumor Immunology Program Site Visit Date: 3/15/7[illegible]
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: $71,400

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Antibodies to Carcinomas in Experimental Animal Models

Principal Investigator: Dr. Lionel Manson
Name/Address: Wistar Institute
Performing Organization: Philadelphia, PA

Contract Number: NO1-CB-43882
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: Development of experimental animal systems for the early detection of antibody response to tumor-associated antigens, as model for problem of antibody detection in patients with carcinomas and other malignant diseases.

Approach: Studies will be performed with primary, autochthonous carcinomas or with carcinomas in early transplant, in inbred strains of mice. A sensitive antiglobulin assay will be used to determine antibody levels.

Progress: The previously used assay in this study, in which adherent live cells were used as the solid phase immunoadsorbent, was found to be unsuitable for a quantitative antibody assay. By binding membrane, antigenic fragments to activated filter paper discs, a solid phase immunoadsorbent was developed with which absolute quantitation was achieved. By using these discs and specific antibody in an inhibition mode antigen can also be measured. Further studies with the spontaneous mammary adenocarcinoma A-10 have confirmed the previous observations that the cell line appears not to be immunogenic in the A/J mouse. Control experiments with an allogeneic tumor have shown that if antibody appeared in the serum during the early growth of the tumor, it should have been detected by the assay used.

Significance to Cancer Research: (NCP Objective 5 Approach 1)

Project Officer: Mrs. Judith Whalen
Program: Tumor Immunology Program Site Visit Date: 2/27/76
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biochemistry and Diagnostic Use of Human Tumor Antigens

Principal Investigator: Dr. Vincent Marchesi
Name/Address Yale University School of Medicine
Performing Organization: New Haven, CT

Contract Number: NO1-CB-43859
Starting Date: 8/8/73 Expiration Date: 11/7/76

Goal: To develop a more discriminating radioimmunoassay for the detection of human carcinoembryonic antigens.

Approach: A new method was developed for the isolation of glycoproteins from human colonic cancer tissue employing detergent extraction and lectin affinity chromatography. Partially purified glycoproteins were chemically cleaved at selective segments of their polypeptide chains, and individual glycopeptides were isolated, purified, and analyzed for their immunological activity.

Progress: Antisera were raised in rabbits to partially purified colonic tumor glycoproteins. Specific antibody populations were isolated by immunoadsorption with purified peptides. Antisera prepared by this approach reacted specifically with extracts from human colonic cancer tissue. Cross reactions were noted with "standard" CEA preparations and the CEA-S prepared by Edgington. A preliminary screening test was carried out with these antisera using human samples provided by NCI. These results suggest that the antisera prepared by this approach may have great specificity for antigenic determinants of human colonic cancer tissue.

Significance to Cancer Research: These results suggest that the development of antibodies to specific antigenic determinants of tumor-associated glycoproteins might be an effective way to produce discriminating antisera which can be used to measure the presence of tumor-associated antigens in human serum.

Project Officer: J. Frederic Mushinski, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunodiagnosis
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

TItle: Synthesis of Cord Factor-Like Components

Principal Investigator: Dr. Walter J. Gensler
Name/Address Boston University
Performing Organization: Boston, MA

Contract Number: NO1-CP-65783
Starting Date: 6/30/76 Expiration Date: 8/31/78

Goal: Preparation of structurally homogeneous cord factor.

Approach: Total synthesis.

Progress: Cord factor has been identified as a substance useful in control of tumors. So far, only material isolated from bacteria has been used for assays. The planned reaction sequence has been successfully brought to an intermediate point, and the work is going ahead.

Significance to Cancer Research: With extraneous matter precluded by this approach, the activity of cord factor can be determined without the possibility of interference or complication by trace impurities that might not be removed from cord factor isolated from natural sources.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: Approx. $70,000

CONTRACT RESEARCH SUMMARY

Title: Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules

Principal Investigator: Dr. Helen Van Vunakis
Name/Address Brandeis University
Performing Organization: Waltham, MA

Contract Number: NO1-CP-23243
Starting Date: 4/20/72 Expiration Date: 1/31/78

Goal: To develop immunoassays that can be used to determine carcinogens, procarcinogens, cocarcinogens, and their metabolites in physiological fluids, tissue extracts and other material. Such assays also facilitate studies on pharmacokinetics, metabolic conversions, and are useful in the characterization of enzyme systems.

Approach: The compound is coupled to a macromolecular carrier that promotes its antigenicity and the resulting conjugate is used for immunization of animals. Resulting antibodies are used to devise radioimmunoassays.

Progress: Radioimmunoassays have been developed for the detection of picomoles of nicotine and its metabolites (cotinine and γ-(3-pyridyl), γ-oxo-N-methyl-butyramide), the NMN, NAD and NADP analogues of nicotine and cotinine, polycyclic aromatic hydrocarbons (benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene); mycotoxins (aflatoxin B_1); the poly-cyclodiene-polychlorinated insecticides (dieldrin and aldrin) and the antineoplastic agents (adriamycin, daunomycin, vinblastine and vincristine). Nicotine and cotinine analogues of NAD and NADP were isolated and identified from incubation mixtures containing nicotine or cotinine, NAD or NADP, and a source of NADase. With radioimmunoassays specific for nicotine and cotinine nucleotides, the existence of the nucleotide analogues was detected in the lungs and liver of rabbits injected with nicotine and cotinine.

Significance to Cancer Research: In addition to monitoring levels of noxious agents in the environment, the availability of such assays facilitates basic studies on carcinogens, procarcinogens and cocarcinogens that may help us understand how they exert their biological activities.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: $88,638

CONTRACT RESEARCH SUMMARY

Title: Tumorigenesis in Tolerant Guinea Pigs

Principal Investigator: Dr. Jerome R. Pomeranz
Name/Address Case Western Reserve University
Performing Organization: Cleveland, OH

Contract Number: NO1-CP-43354
Starting Date: 2/15/72 Expiration Date: 10/14/77

Goal: (1) To study the role of carcinogens with both contact sensitizing and carcinogenic properties on the induction of tumors in animals that have been previously sensitized or rendered tolerant to the carcinogen by a variety of means. (2) To study the induction of primary mammary tumors following the infusion of DMBA into guinea pigs and to investigate the effects of complete Freund's adjuvant (CFA) administration at different times, and doses on the induction of tumors in guinea pigs.

Approach: Tolerance was induced by multiple gastric feedings of dimethylbenz(α) anthracene (DMBA). Sensitization was by topical application of DMBA. Guinea pigs received infusions of DMBA to induce mammary tumors.

Progress: Studies on the effect of contact sensitization and immune tolerance to DMBA on oncogenesis in guinea pigs were completed or have been terminated. When 20 mg DMBA was injected, mortality, presumably due to toxicity, was very severe. However, among survivors, there was little or no difference in the tolerized, sensitized or control groups. When 7 mg was injected, tolerized animals had a roughly comparable rate of tumor formation to controls. Data for sensitized groups are not available.

The observation that femoral vein infusion of guinea pigs induces mammary tumors which have been interpreted as adenocarcinomas has been reconfirmed. Experiments are continuing to determine if the tumors are transplantable. The latency period of the tumors is about one year.

Significance to Cancer Research: The studies outlined above represented a direct approach to the investigation of the immune response following the administration of a chemical carcinogen, and might be relevant to clinical situations in human medicine.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Study Effects of Carcinogens on the In Vitro Synthesis of Complement Components

Principal Investigator: Dr. Harvey Colten
Name/Address: Children's Hospital Medical Center
Performing Organization: Harvard Medical School
Boston, MA

Contract Number: NO1-CP-45612
Starting Date: 6/1/73 Expiration Date: 5/31/77

Goal: To define the effects in vivo and in vitro of chemical carcinogens on several macrophage-mediated functions, including biosynthesis of complement, phagocytosis, cell surface receptors and response to chemotactic factors.

Approach: Guinea pigs are treated with carcinogens such as DMN and DEN. At appropriate times after administration, measurements are made of the effect of the carcinogens on in vitro and in vivo immunologic parameters.

Progress: Among the nitrosamines known to induce tumors several, including diethylnitrosamine and dimethylnitrosamine, are effective inhibitors of guinea pig complement biosynthesis in vivo but not when administered to cells in vitro. In vivo both compounds have a significant (>50% inhibitory) effect on the capacity to generate chemotactic factors in serum of experimental animals. In addition, the capacity of serum to opsonize is markedly inhibited by administration of DEN or DMN to guinea pigs. The effects of DEN on macrophage function are manifest within 24 hours at high doses of carcinogen and within months at low dose.

Significance to Cancer Research: This work provides insight into a number of basic alterations in effector cells of the immune system resulting from exposure to chemical carcinogens. These changes may help to define the causes of cancer and also may lead to the development of an in vitro screening test for carcinogenic potential.

Project Officer: Dr. Tibor Borsos
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Induction of Colonic Tumors in Guinea Pigs

Principal Investigator: Edwin J. Andrews
Name/Address Cornell University
Performing Organization: Ithaca, NY

Contract Number: NO1-CP-65744
Starting Date: 9/30/76 Expiration Date: 10/31/78

Goal: To produce carcinomas of the colon in approximately 700 NIH guinea pigs and subsequently test the immunotherapeutic effect of BCG vaccines versus other modalities of treatment.

Approach: N-methyl-nitrosourea by intrarectal installation is utilized in the production of colon carcinomas. Once animals have tumors, vaccine is introduced through surgical exposure of the affected portion of colon.

Progress: This is a new contract which is not yet fully underway owing to the necessity of procurement of biohazard containment facilities and the long period necessary for colon tumor production following carcinogen administration.

Significance to Cancer Research: The guinea pig offers a valuable model in that the response of its immune system to immunotherapeutic modalities is quite similar to that observed in man. This fact coupled with the ability to produce colon carcinomas in this species has made it the model of choice for studying this disease.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Response of Peripheral Blood Monocytes to Chemotactic Factors

Principal Investigator: Dr. Ralph Snyderman
Name/Address Duke University Medical Center
Performing Organization: Durham, NC

Contract Number: NO1-CP-33313
Starting Date: 6/25/73 Expiration Date: 6/24/77

Goal: To determine if monocyte function is abnormal in individuals with cancer and, if so, to determine the significance of this abnormality to prognosis.

Approach: Humans with cancer or with benign tumors are tested for blood monocyte chemotactic responsiveness before and after surgery. Animals are given neoplastic cells or products thereof and then tested for their ability to mobilize macrophages to inflammatory sites in vivo. Chemical carcinogens are tested for their effects on macrophage function. Inhibitors of monocyte-macrophage chemotaxis are being looked for in humans and in animals with cancer.

Progress: Approximately 60% of patients with various types of cancer have abnormal monocyte chemotactic responsiveness (MCR) in vitro. Melanoma patients with abnormal chemotaxis have a poor prognosis. Abnormal chemotactic responses normalized within weeks after surgical removal of malignant tumors indicating that a neoplasm itself might affect the host's MCR. Subsequent studies in mice have shown that macrophage accumulation in vivo, as well as MCR in vitro, was depressed in animals during the early phases of implanted tumor growth. This depression of macrophage function could be attributed to a factor contained in murine neoplasms which, when given to normal mice, was extremely potent in depressing peritoneal macrophage accumulation and chemotaxis but, paradoxically, enhanced phagocytosis. The serum of tumor-bearing mice also contained potent inhibitor activity for macrophage accumulation. Granulocyte accumulation in vivo and chemotaxis in vitro were not depressed by the presence of a neoplasm or the administration of the factor from neoplasms. By releasing factors which depress macrophage migratory function, neoplasms may protect themselves from immunologically mediated host destruction during the early phases of tumor growth.

Significance to Cancer Research: These data suggest that clinically apparent neoplasms may be those which have developed means to abrogate the host's macrophage-mediated surveillance. If this is the case, then by isolating and characterizing the factor(s) produced by neoplasms which depress macrophage function, we may be able to develop new and unique methods to control cancer development and spread.

Project Officer: Dr. Edward Leonard
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Procurement of Sulfolipids from Mycobacterium tuberculosis Strain H37RV

Principal Investigator: Dr. Mayer B. Goren
Name/Address
Performing Organization: National Jewish Hospital Research Center
Denver, CO

Contract Number: NO1-CP-65746
Starting Date: 6/8/76 Expiration Date: 6/7/78

Goal: To provide quantities of purified sulfatides and of incompletely characterized polar acidic lipids of Mycobacterium tuberculosis H37RV to be screened for antitumor activity.

Approach: In the search for specific components of mycobacteria with antitumor activity, trehalose-6,6'-dimycolate (cord factor, "P3" of E. Ribi) has emerged as a seemingly necessary product which confers activity onto otherwise inactive cofactors such as degraded BCG cell wall or on certain endotoxins, when these latter are associated with droplets of mineral oil in aqueous emulsion (Ribi). These systems have been found effective in prophylaxis and therapy in the line 10 tumor model in Strain 2 guinea pigs. The thesis that cord factor provides only a physical requirement for the necessary association with oil -- one which may be met by a host of agents with appropriate surface activity -- is being tested with the sulfatides and other polar lipids of M. tuberculosis.

Progress: This year's efforts have been largely devoted to the isolation of a sufficient quantity of a variety of sulfolipids from M. tuberculosis. Animal studies have demonstrated that the rare SL-III and synthetic "pseudocord factors" may effectively substitute for P3. If the abundant SL-I-SL-II products are also found to be active, these are more readily available than cord factor; while the activity of "pseudocord factors" encourage us to synthesize for screeniing even simpler products which increasingly depart from the cord factor structure.

Significance to Cancer Research: These studies may define the role of cord factor in antitumor activity and allow utilization of inexpensive, easily synthesized substances as substitutes for exotic natural products which are much more difficult to obtain.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Chemical Carcinogenesis and Immunity

Principal Investigator: Dr. Bruce Zwilling
Name/Address Ohio State University Research Foundation
Performing Organization: Columbus, OH

Contract Number: NO1-CP-53329
Starting Date: 3/25/75 Expiration Date: 3/24/78

Goal: To study the effects of low levels of chemical carcinogens on normal immunological functions of Sprague-Dawley rats.

Approach: Rats are treated with low levels of methylnitrosourea or its noncarcinogenic analog diphenylnitrosoamine. Natural antibody levels to several bacterial antigens are being assessed. The functions of lymphocytes and macrophages are being assessed.

Progress: All animals receiving 4.5 mg/kg MNU and surviving six months or longer developed hyperplastic and later neoplastic lesions in the nonglandular portion of the stomach. Changes in immunological reactivities occurred most consistently in animals receiving the highest carcinogen dose. Alterations occurred in antibody levels, peripheral blood differentials, spleen weights and the percent B cells in the spleen. Other alterations concerned the linearity of the response; for example, the response of macrophages to migration inhibitory factor was nonlinear, increasing late in the study. Most observed or apparent alterations occurred at about the same time as the carcinogen treatments were completed. This also corresponded closely to the time of the appearance of malignant tumors. Present results using a slightly higher carcinogen dose also indicate that the carcinogen has no apparent effect on the immunological reactions being studied.

Significance to Cancer Research: This work will provide insight into basic alterations in effector cells of the immune system resulting from persistent exposure to low levels of chemical carcinogens.

Project Officer: Dr. Tibor Borsos and Dr. Virginia Dunkel
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: $132,605

CONTRACT RESEARCH SUMMARY

Title: Chemical Carcinogenesis and Immunity

Principal Investigator: Dr. Abraham G. Osler
Name/Address Public Health Research Institute of the
Performing Organization: City of New York
New York, NY

Contract Number: NO1-CP-55679
Starting Date: 3/25/75 Expiration Date: 3/23/78

Goal: To study the effects of repeated exposure to low doses of a chemical carcinogen on the host's natural antibody and complement levels.

Approach: Sprague-Dawley rats received biweekly feedings of one of three dosages of the chemical carcinogen N-nitrosomethylurea for a period of about 9 months. A fourth group of untreated rats served as controls. The animals were bled at periodic intervals for serum assays of hemolytic antibody and complement component activities.

Progress: The goal set for this contract will be fulfilled upon completion of the immunologic assays. Progress can be recorded since the multiple biweekly administration of the carcinogen seems to have exerted minimal or no influence on the host's humoral immune system as judged by studies of the reagents mentioned above.

Significance to Cancer Research: When completed, the data should indicate whether repetitive exposure to small amounts of environmental carcinogens attenuates those aspects of the host's immune status which are dependent upon a fully active complement system.

Project Officer: Dr. Tibor Borsos
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: $79,433

CONTRACT RESEARCH SUMMARY

Title: Induction of Malignant Melanoma in Guinea Pigs

Principal Investigator: Dr. Wallace Clark
Name/Address Temple University School of Medicine
Performing Organization: Philadelphia, PA

Contract Number: NOI-CP-33200
Starting Date: 10/1/72 Expiration Date: 12/31/77

Goal: The induction of malignant melanoma in the guinea pig in order to study the developmental biology of a primary neoplasm in relationship to metastases.

Approach: This inductive agent was DMBA applied to the backs of both ears and both flanks. The animals were photographed at regular intervals and all stages of development were studied by photography, histology, and fine structure.

Progress: Malignant melanoma has been induced in several guinea pig strains. The development of the induced changes in the epidermal melanocytic system has been documented over a time span of 2-150 weeks. The tumors are characterized by widespread metastatic disease and their developmental biology is analogous with a number of human and animal tumor systems.

Significance to Cancer Research: Provides a dependable model for the induction of malignant melanoma in an animal model.

Project Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: $129,795

CONTRACT RESEARCH SUMMARY

Title: Studies on an In Vivo/In Vitro System as a Potential Bioassay

Principal Investigator: Dr. Robert Strunk
Name/Address University of Arizona
Performing Organization: Tucson, AZ

Contract Number: NO1-CP-65778
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To investigate and standardize this in vivo/in vitro guinea pig macrophage system using a series of known chemical carcinogens run in parallel with non-carcinogenic analogues.

Approach: Young adult Hartley guinea pigs (GP) are injected intramuscularly (IM) with known chemical carcinogens, their non-carcinogenic analogues, or solvent controls. Factors which could influence the in vitro response route of administration, dosage, strain, sex, and the age of the animal at the beginning of the test have been carefully considered in the experimental design. A peritoneal exudate (PE) is induced in each GP by intraperitoneal (IP) injection of 7 ml of a sterile 3% starch solution. Ninety-six hours after the IP injection, PE cells are harvested, washed and tested for capacity to synthesize the second (C2) and fourth (C4) components of complement (C). Total protein synthesis is also measured.

Progress: Studies were carried out on normal GP and GP injected IM with potential solvents for the carcinogens. None of the 6 solvents used decreased C synthesis. A known carcinogen, dimethylnitrosamine (DMN), and its noncarcinogenic analogue, diphenylnitrosamine (DPN), were used to establish the appropriate methods for detection of carcinogenicity. C2 and C4 synthesis by PE cells collected 144 hours after injections of DMN was decreased by approximately 80% when compared to synthesis by control cells, whereas PE cells from DPN injected animals was decreased by only 10%. The effect was maximal when the carcinogens were injected 48 hours prior to the induction of the PE, with significantly less of an effect seen with only 24 hr. The LD50s of the carcinogens after the IM injection into the GP are also being established for the carcinogens.

Significance to Cancer Research: Standardization of this assay system would make it available for the identification of compounds that may be carcinogenic to humans.

Project Officer: Dr. Tibor Borsos and Dr. Virginia Dunkel
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: Approx. $90,000

CONTRACT RESEARCH SUMMARY

Title: Development of In Vitro Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens

Principal Investigator: Dr. Daniel E. Thor
Name/Address: University of Texas
Performing Organization: San Antonio, TX

Contract Number: NO1-CP-43353
Starting Date: 2/16/72 Expiration Date: 2/15/77

Goal: Development of in vitro methods for the detection of cell-mediated immunologic reactivity to chemical carcinogens.

Approach: Guinea pigs are sensitized to known chemical carcinogens and their lymphocytes are tested in vitro for the release of migration inhibition factor (MIF) after interaction with appropriately conjugated carcinogen.

Progress: A variety of approaches have led to positive findings but the sensitivity and specificity of the reactions precluded the use of MIF release as a routine method for detecting immunologic reactivity to chemical carcinogens.

Significance to Cancer Research: If successful, these studies will make it possible to determine whether human individuals have been exposed to a variety of agents known to be carcinogenic in animals and suspected of being carcinogenic in man. The development of an immunogenic carcinogen-carrier complex with specificity might also be very useful in surveillance studies. Significant differences among various populations in their immunologic reactivity to known carcinogens might make it possible to correlate the degree of reactivity with the subsequent development of cancer. Correlations of this kind might provide the impetus to remove the agents from the environment.

Program Officer: Dr. Herbert Rapp
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy (C&P)
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Intrapleural BCG after Primary Surgery for Lung Cancer

Principal Investigator: Dr. Martin F. McKneally
Name/Address Albany Medical College
Performing Organization: Albany, NY

Contract Number: NO1-CB-53940
Starting Date: 6/1/75 Expiration Date: 5/31/78

Goal: To conduct preclinical and clinical evaluation of the therapeutic efficacy of intrapleural BCG with or without tumor antigen preparations in lung cancer.

Approach: Following surgical resection of their tumors, patients with lung cancer were randomly assigned to intrapleural BCG plus INH, or INH alone. In animal experiments, prior to inoculation with a methyl cholanthrene-induced tumor, mice were treated with intrapleural BCG, or INH, or saline, or intrapleural BCG plus INH.

Progress: Stage I lung cancer patients treated with intrapleural BCG plus INH had a decreased incidence of tumor recurrence and an increased survival time when compared to patients treated with INH alone. Patients with Stage II and III lung cancer derived no substantial benefit from intrapleural BCG. New clinical protocols are being devised. In the mouse tumor experiments, INH exhibited no prophylactic effect of its own, but appeared to augment the prophylactic effect of intrapleural BCG.

Significance to Cancer Research: This randomized controlled trial shows a statistically significant treatment effect due to immunotherapy. A repeat trial with larger numbers of patients will be required to confirm the result.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $90,000

CONTRACT RESEARCH SUMMARY

Title: Augmentation of Tumor-Specific Immunity with Ribonucleic Acid (RNA)

Principal Investigator: Dr. Peter J. Deckers
Name/Address Boston University
Performing Organization: Boston, MA

Contract Number: NO1-CB-43949
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: The development of an in vivo immunotherapy model utilizing ribonucleic acid extracted from the lymphoid tissues of animals immunized with the particular murine tumor being studied.

Approach: Inbred mice bearing immunogenic carcinogen-induced tumors were studied. Tumors were injected into the mice and 5 days later they were treated with ribonucleic acid extracted from the lymphoid tissues of guinea pigs immunized with these particular murine tumors. Treatment continued every other day for 10 days. The growth of the tumor in the leg was then observed as a marker of the immunologic effect of immune RNA. Inbred rats bearing spontaneous mammary tumors known to metastasize to the lungs were treated by amputation of the primary tumor. The micrometastases in the lungs were treated by injecting these rats with RNA extracted from the lymphoid tissue of guinea pigs immunized with these rat tumors. Some rats also received cytoxan in an attempt to decrease their tumor burden. These rats represent a chemoimmunotherapy model.

Progress: In several experiments there has been a decreased incidence of tumor development in mice treated with xenogeneic immune RNA but this decrease in tumor development was only transitory and eventually all mice developed tumors at the same rate and the tumors grew progressively in a similar manner. No prolongation of life was ever demonstrated. In the rat model no statistically significant effect of immune RNA in prolonging the life of rats with metastatic mammary cancer has ever been demonstrated.

Significance to Cancer Research: Immune RNA is known to mediate the transfer of tumor and transplantation immunity and is effective in vitro in augmenting the immunoreactivity of normal lymphocytes and lymphocytes from animals with growing tumors against the tumor-specific antigens of these tumors. It was hoped that this would be an ideal method of immunotherapy in man, but to date no consistent and reliable in vivo animal model has been developed. It is possible to affect the transplantation of tumor isografts with immune RNA but once these isografts have taken sufficiently, then immunotherapy with RNA has not been consistently effective.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Induced Thyroiditis as an Immunotherapeutic Approach to Thyroid Cancer

Principal Investigator: Dr. Paul LoGerfo
Name/Address Columbia University
Performing Organization: New York, NY

Contract Number: NO1-CB-64048
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To evaluate the usefulness of induced thyroiditis as an immunotherapeutic approach to the treatment of thyroid cancer in humans.

Approach: Human thyroid cancer specimens will be studied for thyroglobulin using immunological staining. Serum thyroglobulins will be measured in patients with varying kinds of thyroid disease including cancer. The initial approach will be the induction of thyroiditis in rats and the evaluation of the effects of induced immunity to thyroglobulin on transplantable rat thyroid carcinomas.

Progress: An immunoperoxidase staining technique was developed for thyroglobulin. Applying this technique to formalin-fixed tissues, it was established that over 95% of differentiated thyroid cancers produce thyroglobulin and that even some undifferentiated forms continue to make thyroglobulin. Analyses of saline extracts from ten thyroid cancers for thyroglobulin have shown that most thyroid cancers produce immunoreactive thyroglobulin of the 19S variety.

A radioimmunoassay for thyroglobulin has been developed and it was shown that recurrent thyroid cancer is associated with elevation of serum thyroglobulin in the athyrotic patient. Postoperative thyroid cancer patients are followed to determine if this assay will be useful in detecting early recurrences. It appears that the assay is more sensitive than radioactive scanning techniques.

Induction of autoimmune thyroiditis in rats has begun, using thyroglobulin purified from rat thyroids which have been chemically altered with haptenic agents. The haptenic agents which have been used for thyroglobulin modification include DNCB, succinic anhydride, arsanilic acid, and sulfanilic acid. Currently, ten groups of 12 animals each are being treated with various immunizing agents and/or immunizing schedules.

Significance to Cancer Research: Thyroglobulin is a specific "tumor" antigen in the athyroid patient. Theoretically, thyroid cancer should be treatable by induction of immunity to thyroglobulin. Animal models are being used to determine the best method of inducing this immunity.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $85,000

CONTRACT RESEARCH SUMMARY

Title: Studies of Immune Stimulants in Patients Receiving Radiation Therapy

Principal Investigator: Dr. John R. McLaren
Name/Address Emory University
Performing Organization: Atlanta, GA

Contract Number: NO1-CB-64061
Starting Date: 7/16/76 Expiration Date: 6/15/78

Goal: To determine whether or not radiation therapy modifies the immunocompetence of patients with lung, head and neck, or cervical malignancies and to determine whether or not aggressive immunotherapy with combined BCG and levamisole may alter any such modifications. By long-term follow-up to determine whether or not such modifications in immunocompetence may correlate with disease-free intervals or survival times.

Approach: T- and B-lymphocyte levels and percentages, lymphocyte cytotoxicity, serum toxicity, *in vitro* lymphocyte transformation by mitogen, and sialic acid determinations will be made on cancer patients at appropriate intervals before and following radiation therapy, and on a control population. Following radiation therapy patients will be randomized into arms with or without immunotherapy. Clinical, laboratory, x-ray, and immunological evaluations will be continued for sufficient intervals to observe disease-free intervals and survival times.

Progress: Preliminary data on 31 patients with head and neck cancer, 18 with lung cancer, and 17 with cervical cancer indicate that in most of the above tests, values in cancer patients are depressed below control values. In a number of tests the T values are less than 0.01. Fewer cases have been analyze following radiation since completion of the radiation treatments takes 4 to 6 weeks. To date, the results in this group indicate that, while radiation further suppresses these values, some will subsequently rise to pretreatment or even to control values. Even though prior to radiation, some patients' lymphocytes may stimulate the growth of target cells, following radiation, with lessening of tumor burden, there is a tendency toward increased cytotoxicity.

Greater numbers are needed before meaningful conclusions can be drawn, particularly for potential benefits of immunotherapy, or to demonstrate any increase in disease-free intervals or survival times.

In recent months necessary approval has been obtained from officials at the Veterans Administration Hospital and patient accrual has been initiated from that patient population.

Significance to Cancer Research: The data corroborate mounting evidence that cancer patients have suppressed immunocompetence, and that radiation further depresses many of these immune parameters. Early data suggest that those patients in whom tumor burden is adequately reduced by radiation will show a return of these values toward pretreatment or normal levels. The project demonstrates an enhancement of immunocompetence by means of adjuvant immunotherapy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $220,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutical Trials with Human Tumors

Principal Investigator: Dr. Karl Erik Hellström
Name/Address
Performing Organization: Fred Hutchinson Cancer Research Center
Seattle, WA

Contract Number: NO1-CB-64018
Starting Date: 10/30/74 Expiration Date: 3/31/78

Goal: To evaluate the clinical effectiveness of BCG scarification plus unblocking plasma versus BCG scarification plus normal plasma in patients with Stage II or III malignant melanoma.

Approach: This approach to immunotherapy is based on the demonstration of so-called "blocking" factors present in the sera of animals and humans with progressive neoplastic disease. These blocking factors can abrogate the cytotoxic effects of host lymphocytes immune to tumor target cells in vitro. Suggestive evidence has been presented that these factors are soluble complexes formed from antigens released by the tumor and antibody formed by the host or free tumor antigen itself. It has been proposed that serum blocking factors may play a role in tumor progression. The demonstration of "unblocking" factors present in the sera of animals or patients who are tumor free has lent additional support to this concept and suggested that the passive administration of serum with unblocking activity may be of potential therapeutic benefit.

Progress: The effect of administration of "unblocking" plasma from normal Black donors was compared to that of normal plasma in Caucasian patients with malignant melanoma. Stage II patients and Stage III patients with no clinically evident disease at randomization received plasma and BCG scarification on alternate weeks. Stage III patients with nonresectable disease received the same immunotherapy, combined with systemic chemotherapy (DTIC). A conventional nonimmunotherapy control group was included in the first year of the study. One hundred eight evaluable patients (Stage II, 58; Stage III, 50) have been studied to date. No difference in progression rate or survival has been observed in patients receiving unblocking plasma compared to control plasma. Similarly, no differences have been observed when patients receiving immunotherapy have been compared with patients in the nonimmunotherapy control group. The numbers of patients included in the study to date have been too small to exclude potential treatment differences of substantial magnitude.

Patient entry on the protocol has been closed. The focus of investigation of the present contract year will be continued immunologic evaluation of these patients.

Significance to Cancer Research: Recent studies have demonstrated a correlation between immunologic reactivity (as determined by an in vitro microcytotoxicity assay) and the patients' clinical course.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 11/18/76
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $182,159

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in Outbred Cat Lymphomas and Leukemias

Principal Investigator: Dr. Myron Essex
Name/Address Harvard College
Performing Organization: Cambridge, MA

Contract Number: NO1-CB-64001
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To see if a specific tumor cell immune response of outbred mammals with naturally occurring leukemia and lymphoma can be sufficiently stimulated or rejuvenated to induce and/or sustain clinical remission.

Approach: The plan is to use specific immunotherapy, directed to the feline oncornavirus-associated cell membrane antigen (FOCMA), with and without chemotherapy. This treatment will be given to cats with induced and naturally occurring thymic lymphoma (TL) and acute lymphoblastic leukemia (ALL). Both passive and active immunotherapy will be employed.

Progress: Previous work indicated that leukemic cells from cats with either naturally occurring or induced leukemia possess specific neoantigens on their membranes. This antigen was named FOCMA and it was shown that serum antibody levels to the antigen were inversely correlated with tumor development and progression. Serologic analysis of field populations of cats strongly suggests that this response represents a natural immunosurveillance mechanism. Large pools of feline antibody to FOCMA were developed and characterized. All pools represent individual cats from which consecutive samples were pooled. Some of the donor animals were laboratory cats that were re-inoculated with their own FOCMA-positive cells that had been surgically biopsied, cultivated, and transformed in vitro. Others were animals identified as high in FOCMA antibody following infection under natural conditions. A ^{51}Cr test was developed which is used to titrate FOCMA antibody and measure cat complement levels. FOCMA antibody is highly lytic for tumor cells in the presence of cat or guinea pig complement. It was determined that antibody levels to the major virus proteins (gp70 and p30) were often discordant when compared to the levels of antibody to FOCMA in the same cat.

Significance to Cancer Research: The cat system is probably the only model where studies with spontaneous lymphoid tumors are both economically and scientifically feasible because sufficient knowledge of the tumor antigen-immune system has been developed.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $79,123

CONTRACT RESEARCH SUMMARY

Title: Tumor-Specific Active Immunotherapy of Squamous Cell Carcinoma of the Lung

Principal Investigator: Dr. Hiroshi Takita
Name/Address Health Research, Inc.
Performing Organization: Buffalo, NY

Contract Number: NO1-CB-64007
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To evaluate the therapeutic effects of immunotherapy with tumor vaccine given adjuvant to curative surgical resection in squamous cell carcinoma of the lung by a prospective randomized study.

Approach: Following lung resection, patients with Stage I and II diseases are randomized to 3 groups: (1) control (no treatment); (2) specific immunotherapy; and (3) nonspecific immunotherapy. The specific immunotherapy group will receive solubilized squamous cell lung carcinoma antigen and complete Freund's adjuvant. The nonspecific immunotherapy group will receive Freund's adjuvant only.

Progress: Lung carcinoma-specific tumor-associated antigen is prepared from surgically excised lung carcinoma pooled according to the cell type. Forty surgically excised lung carcinomas have been harvested for preparation of lung carcinoma antigens for immunotherapy and immunologic evaluations. Sixteen patients with squamous cell carcinoma of the lung have entered the study. In addition, 8 lung cancer patients of other cell types have been randomized for the study. Of 24 patients, 6 patients are in the control group, 9 patients in tumor vaccine group, and 9 in Freund's adjuvant group. There has been no death due to recurrence at this time.

Significance to Cancer Research: There is no known effective adjuvant therapy to improve the results of surgery in lung cancer. Phase III study should evaluate definitively the effectiveness of adjuvant tumor-specific active immunotherapy in lung cancer.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $130,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Murine Leukemias Using Lymphoid Cells Sensitized to Leukemia Antigens

Principal Investigator: Dr. Eliezer Kedar
Name/Address Hebrew University
Performing Organization: Jerusalem, Israel

Contract Number: NO1-CB-64050
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To develop an experimental model for the immunotherapy of leukemia (and possibly of other malignancies) using conventional therapeutic modalities in conjunction with adoptive transfer of lymphoid cells sensitized in vitro to tumor cells.

Approach: Lymphoid cells from normal and tumor-bearing mice are stimulated by inactivated leukemic cells under carefully defined tissue culture conditions. The sensitized lymphoid cells are then assessed for their capacity to specifically destroy tumor cells in vitro and to eradicate leukemia implants in vivo.

Progress: (1) Improved tissue culture techniques have been established to generate large numbers of anti-leukemia cytotoxic cells. (2) The induction in vitro of cytotoxic reactivity to poorly immunogenic tumor cells has been markedly amplified by employing in the cultures chemically modified tumor cells and immunopotentiating agents. (3) The in vitro generated sensitized cells have been shown to specifically prevent leukemia development in vivo. (4) A high proportion (over 60%) of mice bearing lethal leukemic isografts were cured using combined treatment of chemotherapeutic drugs and cytotoxic lymphoid cells sensitized in vitro.

Significance to Cancer Research: This study has already demonstrated the efficacy of in vitro sensitized lymphoid cells in impeding tumor growth in vivo. It is expected that this approach could also be applied to the treatment of human malignancies.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunobiology
FY 77 Funds: $26,656

CONTRACT RESEARCH SUMMARY

Title: Preclinical Studies on Tumor Protective Activity of MER

Principal Investigator: Dr. David W. Weiss
Name/Address Hebrew University
Performing Organization: Jerusalem, Israel

Contract Number: NO1-CB-02208
Starting Date: 6/29/74 Expiration Date: 8/28/77

Goal: To develop basic information on the parameters, limits, and mechanisms of the immunomodulating and anti-tumor activities of the MER fraction of killed tubercle bacilli, to provide the necessary baseline for continued application of MER to the treatment of cancer patients.

Approach: Studies are conducted in a variety of animal model systems to ascertain the conditions of maximum MER anti-tumor prophylactic and therapeutic capacities. Parallel studies are being conducted in vivo and in vitro toward definition of the immunological functions (T, B, and K cell, macrophage) which are altered by MER.

Progress: The range of MER prophylactic and therapeutic effects has been established in several distinct tumor test systems, including Rous sarcomas of chickens, line 10 hepatomas in strain 2 guinea pigs, and several syngeneic carcinomas and sarcomas of inbred mice. Under appropriate circumstances of dosage, timing, and especially of cojoint therapy by drugs and/or irradiation, MER is capable of pronounced and highly significant effects in heightening host resistance. MER is a polyclonal B-cell mitogen and a potentiator of specific antibody formation to defined antigens. It also stimulates a number of defined T-cell and macrophage functions, and it protects intact animals against the immunosuppressive effects of a variety of agents, including cancer chemotherapeutic drugs. Mode of action studies indicate that MER acts directly on immunocytes, in addition to any effects on the circumstances of sensitization.

Significance to Cancer Research: MER has already been shown to be a potentially useful immunotherapeutic agent in the treatment of malignancy in man. The information obtained from animal studies will facilitate its effective use in man more effectively, and also cast light on fundamental principles of immunotherapy in general.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 9/2/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Preparation and Distribution of Rabbit Serum Complement

Principal Investigator: Dr. Marianna Cherry
Name/Address Jackson Laboratory
Performing Organization: Bar Harbor, ME

Contract Number: NO1-CB-53870
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To determine whether any inbred strains of rabbits produce a serum with a consistently high level of serum complement and, if so, to investigate ways of making this reagent available to the research community for testing normal and malignant cells.

Approach: To screen sera from 10 rabbits of each sex from each of 10 inbred or partially inbred rabbit strains in our colony by complement-dependent cytotoxic assays, first with mouse target cells (normal and malignant) and then, in cooperation with other laboratories, with human cells of normal or malignant origin.

Progress: Complement-dependent cytotoxic assays are widely used in studies of human cells, both normal and malignant, for identification of antigens of the cell membrane, particularly those specific for malignant cells. Complement, a collective group of serum proteins, is an absolutely requisite reagent for such tests, and must be both high in specific activity and low in nonspecific toxicity for the cells to be tested. Among random-bred rabbits, the species of choice, only 1 animal in 40 produces acceptable complement by these criteria. By tests with normal and malignant cells from mice, it was shown during the first full year of this contract that certain inbred strains of rabbits consistently produce better complement than others. Preparations are now under way for sending selected samples of rabbit serum to several outside investigators for complement assays on human cells.

Significance to Cancer Research: Alteration of cell membrane antigens generally accompanies transformation from normal to neoplastic cells. Studies of these antigens are difficult technically, the more so due to the lack of a reliable source of complement. If certain inbred rabbit strains do, as we believe, provide high specific complement levels, the many ongoing projects in this area should be materially assisted.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: The Immunological Stimulant Properties of Amphotericin B

Principal Investigator: Dr. J. Russell Little
Name/Address Jewish Hospital of St. Louis
Performing Organization: St. Louis, MO

Contract Number: NO1-CB-43872
Starting Date: 3/1/75 Expiration Date: 2/28/77

Goal: To evaluate immunologic responses in animal models receiving Amphotericin B (AmB).

Approach: Groups of animals were immunized with a particular antigen (SRBC, a hapten-protein complex or a contact skin sensitizer) with or without coadministration of Amb. Quantitative assays of immune responsiveness were used to compare results in the two groups. Humoral immunity was measured by hemolysin in gel assay; cellular immunity was measured by the Simonsen splenic index assay of GVH reactions as well as the ^{125}I-UdR ear uptake assay of delayed hypersensitivity.

Progress: It had been shown previously that AmB and BCN4 had synergistic effects in the treatment of advanced disease due to a transplantable AKR leukemia. This combination therapy resulted in 40-70% cures as determined by a leukemia cell clonogenic assay and by long-term survival and resistance to rechallenge of the treated mice. AmB has been demonstrated to be a potent immunostimulant in mice that augments both cell-mediated as well as humoral immunity to several antigens. The magnitude of its immunostimulant effects appears to be under genetic control.

Significance to Cancer Research: The results indicate clearly that amphotericin, a crystalline, homogeneous compound which has been used in man (as an antifungal agent) for two decades, has potent immunoadjuvant effects. The feasibility of its use in the treatment of cancer is suggested by its use in achieving cures in mice bearing a very large body burden of leukemia cells.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Test Device with Standardized Antigens to Assay Delayed Hypersensitivity

Principal Investigator: Gary L. Hein
Name/Address Lincoln Laboratories, Inc.
Performing Organization: Decatur, IL

Contract Number: NO1-CB-43954
Starting Date: 6/30/74 Expiration Date: 7/29/77

Goal: To develop a multi-test device to be used in studying skin test responsiveness in patients receiving immunotherapy.

Approach: Standardized viral, bacterial, and fungal antigens will be developed in concentrated form which will permit their use on a multi-puncture skin test device. The device will be constructed so that multiple antigens may be applied simultaneously in a highly reproducible manner.

Progress: The device which simultaneously administers eight different antigens has been produced, and the following antigens have been developed: Streptococcus, Candida, Trichophyton, Proteus, Diptheria toxoid, tetanus toxoid, and tuberculin. With the exception of tuberculin, each antigen in the test battery was selected for its anticipated ability to elicit a positive reaction rate of 50% or greater in healthy subjects. Safe and effective dose ranges for each antigen were first established in sensitized guinea pigs using different dilutions. The optimal test dose for each antigen, that is, the minimal dose needed to cause a significant delayed hypersensitivity skin test reaction in a maximum number of healthy individuals, will be or has been established in human volunteers. Standardization by delayed cutaneous hypersensitivity units of each antigen from one production lot to another is being accomplished. It is expected that testing of the device and antigens in cancer patients will be undertaken shortly.

Significance to Cancer Research: By providing cancer researchers with a battery of standardized skin test antigens and a testing device that allows uniform administration and reproducible results, it is hoped that more accurate and convenient assessment of cell-mediated immunity will be possible, aiding in diagnosis, prognosis, and treatment selection.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Dates: 7/9-10/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $103,744 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Squamous Cell Carcinoma of the Lung Treated by Resection or Radiotherapy

Principal Investigator: Dr. Engracio P. Cortes
Name/Address Long Island Jewish-Hillside Medical Center
Performing Organization: New Hyde Park, NY

Contract Number: NO1-CB-64064
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: To determine the efficacy of MER in Stages I and II squamous cell carcinoma of the lung (SCC) treated by resection and/or radiotherapy and to determine the efficacy of MER vs. vincristine + adriamycin + cyclophosphamide (VAC) vs. MER + VAC in Stage III SCC treated with radiotherapy with or without prophylactic brain irradiation.

Approach: Randomization is done according to stage stratification:

Stage I (completely resected)	Stage III (RT)	
Arm I - No MER	Arm I - MER	)
Arm II - MER	Arm II - MER	) ± Brain RT (Randomized)
Stage II (completely resected)	Arm III - MER + VAC	)
Arm I - No MER		

Progress: Eight patients who had undergone surgery (Stages I and II) and radiotherapy (Stage II only) for squamous cell carcinoma of the lung were randomized to receive the following treatment arms: Arm I (No MER), 4 patients; Arm II (MER), 5 patients. Twelve patients with Stage III SCC of the lung, previously treated with radiotherapy, were randomized into 3 treatment arms with or without prophylactic brain irradiation: Arm I (MER alone), 5 patients; Arm II (VAC), 4 patients; and Arm III (MER + VAC), 3 patients. Each of the above treatment arms was given every 4 weeks for 12 courses. The administration of either MER, VAC, or MER + VAC so far is tolerable. Baseline immunologic studies were done at the time patients were placed on protocol. Of eight patients in Stage I and II, five showed positive skin hypersensitivity reactions to two or more antigens. One patient was positive to only one antigen. Five of eight patients in Stage III reacted well to two or more antigens but two of eight patients had no reaction to any of the four test antigens used. Average lymphocyte T-cell values decreased with more advanced disease when each group was compared against the other. The differences in B-cell percentage values between stages of presentation were less clear. With continued MER therapy increasing skin activity was seen.

Significance to Cancer Research: The study is ongoing and it is too early to draw further conclusions.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $105,000

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Responses

Principal Investigator: Dr. Michael J. O'Connell
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-53874
Starting Date: 6/1/75 Expiration Date: 5/31/78

Goal: To evaluate the effects on immunologic determinants, therapeutic effects, and toxicity of BCG-Tice strain (lyophilized) versus BCG-Pasteur strain (lyophilized) versus BCG-Pasteur strain (fresh frozen) in patients with advanced cancer.

Approach: Patients, stratified by performance status and reactivity to recall antigen skin tests, are randomized on a double blind basis to one of the three BCG-preparations or to a placebo. Treatments are given by the tine technique on either weekly or monthly schedules, depending upon distance from the Mayo Foundation. Serial assessments of several immune parameters are made.

Progress: As of December 1, 1976, 52 evaluable patients have been entered on study. No serious toxic reactions to BCG have been observed. Primary toxicity has been mild to moderate local cutaneous inflammation at the site of BCG applications. None of the BCG preparations in the dose, routes, and schedules of administration employed has resulted in significant improvement of immune status of patients with advanced cancer as compared to the placebo group on the basis of the tests used. Inadequate numbers of patients have been studied as yet to adequately compare the individual BCG preparations or administration schedules. The current protocols include previously untreated patients with asymptomatic Duke's D colorectal cancer and advanced cancer patients refractory to cytotoxic chemotherapy.

Significance to Cancer Research: The value of using the patient with advanced refractory cancer as a model for the study of immune stimulants will be determined. Identification of a more potent and/or less toxic form of BCG preparation would have practical clinical applications to future clinical trials involving BCG immunotherapy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee of Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Human Cancer

Principal Investigator: Dr. Evan Hersh
Name/Address M. D. Anderson Hospital & Tumor Institute
Performing Organization: University of Texas System Center Center
Houston, TX

Contract Number: NO1-CB-33888
Starting Date: 10/25/74 Expiration Date: 6/29/77

Goal: To develop immunological treatments (immunotherapy) for patients with human cancer.

Approach: Immunotherapy -- Nonspecific, specific as well as immunorestorative approaches are being applied. In the area of nonspecific immunotherapy, the major emphasis has been on the use of microbial vaccines, particularly BCG and nonviable extracts of BCG. Other killed microbial organisms were also used including Corynebacterium parvum and pseudomonas vaccine; tumor cell vaccines, using unmodified and modified tumor cells, and purified tumor antigens; and immunorestorative agents including levamisole and thymosin. Immunotherapy is applied after surgical reduction of metastatic disease. Thus, adjuvant immunotherapy is being combined with chemotherapy for patients with microscopic and disseminated disease.

Progress: Past results demonstrated that (1) adequate numbers of viable Tice BCG organisms could prolong the postoperative disease-free interval and survival of patients with recurrent melanoma in regional lymph nodes; (2) BCG combined with chemotherapy could prolong the duration of remission and survival of patients with disseminated melanoma compared to chemotherapy alone, and (3) BCG added to chemotherapy prolonged remission and survival of responders in AML but not ALL. In the present contract year, BCG added to triple drug chemotherapy (FAC) prolonged durations of remission and survival of patients with metastatic breast cancer. The use of triple drug combination FAC plus BCG postoperatively prolonged the disease-free interval and survival of patients with Stage II and III breast cancer compared to surgery alone. Preliminary data suggest that the use of an intravenous immunostimulant, C. parvum, potentiates response rates for patients with advanced melanoma receiving DTIC or DTIC plus actinomycin D. Phase I development of pseudomonas vaccine, thymosin, levamisole, and MER has been completed.

Significance to Cancer Research: Immunotherapy could have far-reaching significance for the eventual prolongation of life and perhaps cure of cancer patients. Patients with minimal amounts of disease could conceivably be cured after major reduction of disease by chemotherapy, surgery, and radiotherapy since immunotherapy has the capacity to work by zero order kinetics. Eventually, immunoprophylaxis programs could be developed for the prevention of cancer.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $906,046

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in the L_2C Guinea Pig Leukemia

Principal Investigator: Dr. Samuel G. Murphy
Name/Address M.D. Anderson Hospital & Tumor Institute
Performing Organization: University of Texas
Houston, TX

Contract Number: NO1-CB-64003
Starting Date: 6/30/76 Expiration Date: 6/30/78

Goal: To use the guinea pig leukemia as an animal model (1) to evaluate those parameters (dosage, route of administration, schedule) that influence the efficacy of immunotherapeutic agents and (2) to provide insights into the principles that govern the structuring of sequential and combination immunotherapy protocols.

Approach: Following cytoreductive therapy for overt leukemia and radiation therapy to the craniospinal axis (XRT) to prevent late CNS relapse, animals are treated with potential immunotherapeutic agents. In randomized experiments, the route, dose and schedule are varied and animals followed with peripheral WBC counts to determine duration of remission or cure.

Progress: The animal model has been reestablished at the M.D. Anderson Hospital and Tumor Institute and a breeding colony of strain 2 guinea pigs has been established at the University of Texas Environmental Science Park at Bastrop, Texas. Experiments have been completed evaluating intraperitoneal, intradermal, and intravenous BCG. Only I.V. BCG has provided suggestive evidence of a therapeutic biological effect and was most effective at a dose of 10^8 organisms given 2 days after XRT. Preliminary studies with Corynebacterium parvum indicate this agent has a therapeutic potential in the L_2C leukemia model which is dose and schedule dependent. At doses of 0.7 mg given weekly, toxicity developed, but at 0.35 mg there was prolongation of remission. These studies represent positive leads which are currently being pursued.

Significance to Cancer Research: This model was developed to investigate the interactions of specific and nonspecific immunotherapy in an animal model whose pathophysiology and therapy closely resemble human acute leukemia. The studies suggest that immunotherapy can prolong remission and will be a useful model to investigate the interaction of immunotherapy agents.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Mechanism of Action of Immunopotentiators

Principal Investigator: Dr. Alan M. Kaplan
Name/Address Medical College of Virginia
Performing Organization: Virginia Commonwealth University
Richmond, VA

Contract Number: NO1-CB-43877
Starting Date: 3/15/75 Expiration Date: 3/14/77

Goal: To evaluate the immunologic effects of immunopotentiators in relation to their immunotherapeutic capacity.

Approach: The immunologic action of selected immunopotentiators will be evaluated in model systems for antibody production and cellular immunity. The changes in these responses toward tumors from tumor-bearing mice will be measured and compared with the model system. In addition, the effectiveness of the immunopotentiators in the treatment of tumors after using surgery or chemotherapy to remove the bulk of the tumor will be evaluated.

Progress: Pyran copolymer, polyacrylic acidmaleic anhydride, and C. parvum are being used for in-depth studies of modes of action of immunopotentiators on two tumor systems: Lewis lung carcinoma and the MCA 2182 fibrosarcoma. Pyran has been shown to enhance primary and secondary IgM and IgG response of mice to sheep erythrocytes in a way that requires T lymphocytes. The leukocyte adherence inhibition assay and tumor cell-induced lymphocyte blastogenesis are in use for evaluation of T-cell function. This work will be concluded in the next contracting period.

Significance for Cancer Research (NCP Objective 4,6 Approach 1,3,4,6)
Understanding the mechanism by which immunopotentiators act is necessary for optimum manipulation of immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 2/21/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Chemoimmunotherapy of Acute Myelocytic Leukemia

Principal Investigator: Dr. James F. Holland
Name/Address Mt. Sinai School of Medicine
Performing Organization: New York, NY

Contract Number: NO1-CB-43879
Starting Date: 6/30/74 Expiration Date: 12/20/77

Goal: To evaluate the usefulness of neuraminidase-treated allogeneic AML cells (VCN-AML) in acute myelocytic leukemia (AML).

Approach: Following remission induction with cytosine arabinoside and daunorubin, AML patients will be randomly assigned to maintenance chemotherapy alone or in combination with VCN-AML. Patients will be studied with both in vivo and in vitro immunologic tests. This new protocol reflects a change in chemotherapy and deletion of MER immunotherapy.

Progress: A total of 54 evaluable patients have been entered in a trial comparing: (1) chemotherapy alone, (2) chemotherapy plus VCN-AML, and (3) chemotherapy plus VCN-AML plus MER. During the period of study (two years) one change in chemotherapy occurred so that the patients fell into two subpopulations. It appeared that patients receiving chemotherapy plus VCN-AML showed a trend toward a more prolonged remission when compared to patients receiving chemotherapy alone. MER added no further benefit and may be detrimental. A new protocol has been initiated which incorporates an additional change in chemotherapy to a more optimal regimen and a deletion of the MER immunotherapy.

Significance to Cancer Research: Suggestive early leads in immunotherapy of AML require that vigorous application of other possible modalities to this disease be undertaken.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 4/5/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $210,631

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in Fibrosarcomas of Chickens

Principal Investigator: Dr. Stephen P. Lerman
Name/Address New York University Medical Center
Performing Organization: New York, NY

Contract Number: NO1-CB-64043
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To evaluate the usefulness of various immunotherapeutic approaches in transplantable and primary carcinogen-induced fibrosarcomas in histocompatible SC strain chickens.

Approach: Methods of inducing specific immunity to individual fibrosarcoma lines in the same strain will be developed optimally with respect to sites, dose and frequency of injections. Particular attention will be given to the use of BCG and C. parvum; however, tumor cell vaccines also will be evaluated. Immunotherapy will be combined with chemotherapy in some studies.

Progress: During the first seven months of this contract, the contractor began to analyze the potent effects of C. parvum (CP) and BCG in facilitating the induction of tumor immunity to transplantable fibrosarcomas in histocompatible chickens. The most successful method of immunization was by simultaneous local injection of live tumor cells and BCG or CP into animals which had received systemic injections of these immunotherapeutic agents previously. Tumor immunity could also be induced in CP-pretreated, BX, agammaglobulinemic chickens by injection of tumor cells and CP. Winn tests with spleen or peripheral blood leukocytes indicated the presence of tumor line specific immunity in T cells of both normal and BX immune birds. Addition to 10^6 tumor cell inocula of spleen cells immune to CP or human γ-globulin did not inhibit tumor growth except when mixed with small doses of the corresponding antigen. Similarly, injection of small doses of antigen into tumors growing in animals with delayed hypersensitivity to this antigen also caused tumor growth inhibition. Results indicate that a host cell component, probably a monocyte-macrophage, was collaborating with donor T cells in the expression of tumor-specific or other antigen-specific immunity leading to local tumor rejection and, in many cases, also to rejection of unmanipulated tumors in a distant site.

Significance to Cancer Research: The information to be gained from such a systematic study is essential in development of immunotherapeutic procedures.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Qualitative Assays of Monocyte-Macrophage Function

Principal Investigator: Dr. Gerald W. King
Name/Address Ohio State University
Performing Organization: Columbus, OH

Contract Number: NO1-CB-53936
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To develop quantitative in vitro assays of human monocyte-macrophage function that will reflect in vivo functional status and that will further define the role of this cell system in immune responses.

Approach: To develop and evaluate for reproducibility various assays of blood monocyte function using normal cells. These assays are then performed on patients with resected malignant melanoma who are receiving adjuvant immunotherapy or patients with disseminated tuberculosis or cancer. This defines the efficacy of the assays in situations of functional stimulation or suppression.

Progress: Normal values for the following monocyte assays have been developed and established: quantitative NBT reduction and superoxide generation; hydroxyl radical generation; technetium sulfur colloid phagocytosis; and monocyte-HeLa cell growth inhibition (cytostatic effect). The effect of methanol extracted residue (MER) of BCG was studied on monocyte chemotaxis, membrane binding of antibody-coated red cells, and in the monocyte-HeLa cell growth inhibition assay. This clinical trial of MER failed to yield any consistent effect on these three assays of monocyte function. New assays being developed are: (1) the quantitation of antibody-dependent monocyte cytocidal activity utilizing human rbc and later malignant cells as the target, (2) an assay for monocyte content and synthesis of lysozyme, a major component of protein synthesis of this cell type, (3) an assay to study the interaction of monocytes with prelabeled target cells (HeLa and other malignant cell lines) to determine level of cytocidal activity.

Significance to Cancer Research: These assays provided information on the basic metabolism and function of the normal monocyte, including insight into its ability to kill tumor cells. Further development will provide a quantitative means of monitoring nonspecific immunotherapy and immunocompetence in cancer patients.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Transfer Factor Therapy of Sarcoma

Principal Investigator: Dr. Albert F. LoBuglio
Name/Address
Performing Organization: Ohio State University Research Foundation
Columbus, OH

Contract Number: NO1-CB-43878
Starting Date: 4/1/74 Expiration Date: 3/31/77

Goal: To evaluate the efficacy of transfer factor therapy of human sarcoma in a blind and randomized study.

Approach: Patients with low tumor mass (post-resection without residual clinical disease) are randomly allocated to receive transfer factor or saline for 7 years. Incidence of recurrence is the end point. Specific and nonspecific in vitro indices of immune status are followed.

Progress: The study continues and the code is unbroken. Due to development of effective adjuvant chemotherapy for sarcomas, patient accrual has closed although initially entered patients continue on therapy.

Significance to Cancer Research: This study should lead to a better understanding of the role of transfer factor in therapy of human malignancy and the value of several in vitro assays (MIF, BT, cytotoxicity) in monitoring treatment.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: New Approaches to Immunotherapy

Principal Investigator: Dr. Jerald J. Killion
Name/Address Oklahoma Medical Research Foundation
Performing Organization: Oklahoma City, OK

Contract Number: NO1-CB-53937
Starting Date: 6/27/75 Expiration Date: 9/26/77

Goal: To improve the use of active, specific immunotherapy of cancer by studying the immunotherapeutic value of isolated tumor cell subpopulations.

Approach: Murine tumor cell subpopulations are isolated by cell-affinity chromatography from lectin-nylon columns. This procedure separates tumor cells based upon their expression of cell-surface carbohydrates. These subpopulations are then used in immunotherapy protocols of L1210 leukemia (following reduction in tumor burden by chemotherapy).

Progress: The studies have shown the following general trends in combination chemotherapy-immunotherapy of L1210 leukemia: (a) cells isolated from either concanavalin A-columns or fucose protein-columns were effective in immunotherapy; and (b) cells isolated from galactosamine-specific columns were deleterious, i.e., the rate of death of mice treated with these subpopulations was faster than those mice treated with chemotherapy only. These studies have also been extended to EL4 lymphoma (combination chemotherapy - immunotherapy). Preliminary results indicate that unlike L1210 leukemia, cells isolated from galactose-specific columns are effective immunogens. Current studies are evaluating the use of tumor cell subpopulations combined with nonspecific immunostimulants, BCG and C. parvum.

Significance to Cancer Research: The present work indicates that specific immunotherapy may be either effective or deleterious, when combined with primary chemotherapy. Attempts to define the use of isolated tumor subpopulations in immunotherapy of residual tumor continue.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $20,000

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Evaluation of Therapy with Neuraminidase-Treated Tumor Cells

Principal Investigator: Dr. G. Mark Kollmorgen
Name/Address Oklahoma Medical Research Foundation
Performing Organization: Oklahoma City, OK

Contract Number: NO1-CB-43864
Starting Date: 11/1/74 Expiration Date: 1/31/77

Goal: To determine the therapeutic effectiveness of specific and nonspecific immunostimulants in tumor-bearing mice after tumor burden had been reduced with chemotherapeutic agents.

Approach: Tumors were transplanted into mice and allowed to grow and metastasize for various intervals. Tumor-bearing mice were then treated with different drugs and doses at various times after tumor transplant. Immunostimulants were given at various intervals with respect to chemotherapy. Nonspecific stimulants included BCG, C. parvum, and reovirus while neuraminidase-treated tumor cells, grown in culture or in mice, were used as specific immunostimulants. The therapeutic value of chemotherapy and/or immunotherapy was determined by the surviving fraction, the mean survival time and the presence of tumor-specific immunity in surviving mice.

Progress: Both specific and nonspecific immunostimulants provided significant therapeutic value in several different animal tumor-host systems when administered under proper conditions. Proper conditions included the drug and dose used to minimize tumor burden, the interval between chemotherapy and immunotherapy, the quantity of immunostimulant, and the route of injection. Mice which were immunosuppressed before immunotherapy were not able to respond to immunostimulants. Immunostimulants which provided value in one tumor-host system were not always effective in other systems. Highly immunogenic cells did not necessarily provide therapeutic benefit and the therapeutic value provided by a nonspecific immunostimulant was compromised in the presence of another nonspecific immunostimulant. Successful therapy correlated with development of tumor immunity as measured by *in vivo* and *in vitro* assays.

Significance to Cancer Research: Immunostimulants have been used to treat a number of patients with different types of cancer. Generally, results have not been encouraging. The studies indicated that the therapeutic benefit of immunotherapy was limited by a number of factors. Unless stimulants were administered under proper conditions, the potential therapeutic benefit was not realized. A predictive relationship between immunological status and probability of a response has not been established.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 5/21/73
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in the Immune Response to Tumors

Principal Investigator: Dr. James L. Krahenbuhl
Name/Address Palo Alto Medical Research Foundation
Performing Organization: Palo Alto, CA

Contract Number: NO1-CB-43873
Starting Date: 5/1/74 Expiration Date: 4/30/77

Goal: To define the role of the monocyte-macrophage system in resistance to cancer through an in-depth study of the ability of human and animal macrophages (MØ), monocytes and monocyte-derived MØ to destroy tumor cells (TC).

Approach: All studies are performed in vitro with alveolar or peritoneal MØ from mice or peripheral blood monocytes and monocyte-derived MØ from human volunteers. A variety of mouse and human TC lines are employed as target cells. Immunological and chemical methods are employed to determine their efficacy in activating MØ to become cytotoxic to TC, the manifestations of MØ cytotoxicity (i.e., cytostatic and/or cytocidal effects) and the mechanisms which underlie them.

Progress: Past studies have shown that activated macrophages (AMØ) were cytotoxic for TC in vitro. Cytokinetic studies showed the importance of the AMØ:TC ratio to the level of cytotoxicity and emphasized the importance of conditions which favored effector to target cell contact. AMØ-mediated cytostatic effects were shown to be spontaneously reversible (i.e., TC escaped and renewed multiplication). In contrast to infection with viable Toxoplasma gondii organisms, activation of MØ by treatment with C. parvum was shown to be temporary and was route and dose dependent. During the past year the cytotoxic effects of AMØ were shown to consist of cytostatic and cytocidal components. All TC were susceptible to cytostatic effects. Some TC were also killed, but as a late event. Some TC were not killed, but AMØ effected a prolonged cytostatic effect. Cytostatic effects of AMØ, in the absence of a cytocidal effect, were manifested both as an inhibition of DNA synthesis and as an inhibition of TC mitosis. Human MØ could be activated to markedly inhibit the multiplication of human tumor cells.

Significance to Cancer Research: Nonspecific agents, such as BCG and C. parvum, are thought to stimulate a population of AMØ. Basic studies have further defined: how AMØ manifest their cytotoxicity for TC, some of the limitations of this effect, and some of the variables which determine the efficacy of activation of MØ in different anatomical compartments.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Treatment of Minimal Residual Systemic Tumor

Principal Investigator: Dr. Gerald L. Bartlett
Name/Address
Performing Organization: Pennsylvania State University
Milton S. Hershey Medical Center
Hershey, PA

Contract Number: NO1-CB-33891
Starting Date: 11/1/74 Expiration Date: 10/31/77

Goal: To develop animal tumor models which will be useful for evaluating immune stimulants as immunotherapeutic agents.

Approach: Four different tumor-host models (guinea pig line 10, mouse LSTRA leukemia, rat 13762 mammary tumor and mouse CaD2 mammary tumor) are being used to assess several different effects of immune stimulants and to avoid conclusions based on model idiosyncrasies. At various times after tumor implantation, animals are given immunotherapy, either alone or in combination with other means of treatment. The effect of the immunotherapy and the role of immune stimulants in immunotherapy are evaluated in terms of improved survival of treated animals. Animals which survive are further tested for improved tumor rejection immunity.

Progress: During the current contract year, it was shown that high doses of irradiated leukemia cells (LSTRA) are immunogenic; BCG functions as a weak adjuvant for subimmunogenic tumor cell doses. Immunotherapy with irradiated cells or with mixtures of irradiated cells in BCG, is partially effective if given while the initial tumor burden is small or during drug-induced remission. C. parvum and MER are effective adjuvants for a tumor-specific vaccine (line 10). Visceral tumor can be successfully treated with i.d. injection of tumor-specific vaccine (line 10). Combination therapy is highly effective for the rat mammary tumor (13762): Surgery after 21 days of tumor growth, followed in three days by chemotherapy, prolongs survival but is not curative; the addition of immunotherapy (either as presurgical intratumor injection or as postoperative i.v. injection of C. parvum are also synergistic in the CaD2 system, but survivors do not have tumor immunity.

Significance to Cancer Research: Caution should be exercised in drawing generalizations about immunotherapy in a single experimental or clinical setting. Results of this study further illustrate the diversity of outcomes that may be anticipated from "immunotherapeutic" procedures and indicate the need for much more information concerning (1) the mechanisms of immune host-tumor interaction in animal and human tumors, and (2) the mechanism of action of effective immunotherapy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 5/30/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $194,130

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Accessible Neoplasms and Analysis of Immune Status In Vivo

Principal Investigator: Dr. Edmund Klein
Name/Address Research Foundation, State University of New York
Performing Organization: Buffalo, NY

Contract Number: NO1-CP-33373
Starting Date: 3/21/75 Expiration Date: 4/20/77

Goal: Exploratory studies in the utilization of topical immunotherapy and correlation with in vivo measurement of immune status.

Approach: Patients with various neoplasms originating or metastasizing to the skin will be entered into specific clinical protocols for application of topical immunotherapeutic agents. Some patients will receive BCG by scarification. Multiple antigens will be used for skin testing on all patients and the skin reactivity will be correlated with clinical status and immunotherapy administration.

Progress: The contractor has developed clinical protocols and entered patients into these protocols as follows: (1) Protocol #438--BCG Effects on Skin Test Responses and Incidence of Recurrence in Surgical Remission of Malignant Melanoma. Forty-seven patients are evaluable in this study and it will be completed in one year. (2) Protocol #69-273--Double Blind Comparison of Topical 5-FU, PPD, and Combination of 5-FU/PPD on Cutaneous Neoplasms. No differences were found among the therapeutic agents and this protocol is now terminated.

Significance to Cancer Research (NCP Objective 2,5,6 Approach 1,3,4)
Detailed information regarding results of skin testing in relation to clinical status and therapy provide vital feedback for therapeutic manipulations.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 10/31/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Macrophage Activation in Tumor Immunity and Immunotherapy of Rat Mammary Tumor

Principal Investigator: Dr. W. Hallowell Churchill
Name/Address Robert B. Brigham Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-33896
Starting Date: 11/1/74 Expiration Date: 10/31/76

Goal: To study the mechanism of tumor immunogenicity in order to design a more effective and rational program of tumor immunotherapy in man.

Approach: The contractor will evaluate the ability of macrophages to kill tumor cells after in vitro activation by lymphocyte mediators, and determine whether macrophages activated by adjuvants in vivo show differences in tumor cell killing activity in vitro. In addition, he will extend these studies to human monocytes.

Progress: The investigators have established new systems for the study of macrophage cytotoxicity for tumor cells that will facilitate dissection of the mechanisms of tumor cytotoxicity and how such mechanisms might be stimulated for immunotherapeutic purposes. Suspension cultures of peritoneal exudate cells activated by lymphocyte mediators appear to kill embryonic fibroblasts and tumor cells to the same extent.

Significance to Cancer Research: (NCP Objective 6 Approach 1,4) Immunotherapeutic manipulations in animals are needed to evaluate multiple parameters of immunotherapy that cannot satisfactorily be tested in humans.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Quantitative Assays of Monocyte-Macrophage Function

Principal Investigator: Dr. John R. David
Name/Address Robert B. Brigham Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-53869
Starting Date: 6/1/75 Expiration Date: 5/31/78

Goal: To determine whether biochemical changes in human monocytes relate to the tumor-killing capacity of these cells.

Approach: Quantitative assays of macrophage function will be developed with guinea pig macrophages and ultimately applied to human macrophages. Changes in monocyte-macrophage surface membranes, metabolism and tumor-killing capacity will be explored.

Progress: Purified human monocytes have been obtained from peripheral blood using a Ficoll gradient centrifugation technique. Potent preparations of human lymphocyte mediators (MIF and MAF) were obtained by enzyme treatment of lymphocytes. In vitro activation of monocytes with MIF/MAF is associated with an increase in the production of plasminogen activator, an increase in the rate of leucine incorporation, an increase in the flux of Ca^{++} across the plasma membrane, and an increased rate of pinocytosis of horseradish peroxidase. Next year, investigators will attempt to correlate biochemical changes in the monocyte with the ability of this cell to kill human tumor cells in vitro.

Significance to Cancer Research: Evaluation of macrophage activity may determine the effectiveness of immunotherapeutic manipulations.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $118,000

CONTRACT RESEARCH SUMMARY

Title: Activated Macrophages as Immunotherapeutic Agents

Principal Investigator: Dr. Willy F. Piessens
Name/Address Robert B. Brigham Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-64057
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To determine if in vitro activated macrophages can inhibit the growth of tumors in vivo.

Approach: Strain 2 guinea pig macrophages are activated in vitro with the lymphocyte mediator, macrophage activating factor, or with endotoxin. The cells are then mixed with tumor cells and the mixture is injected subcutaneously onto nonimmune animals.

Progress: Activated macrophages inhibit the local growth of the syngeneic tumors, line 1 and line 10 hepatoma, when mixed with tumor cells in the ratio of 3 macrophages per tumor cell or more. The higher this ratio, the better the therapeutic effect. So far, we have only been able to delay tumor growth but have not been able to suppress tumors completely. Studies are being undertaken to manipulate the macrophage cell surface in such ways as to increase this cell's cytotoxic capacity in vivo and in vitro.

Significance to Cancer Research: Our experiments are designed to explore a new approach to nonspecific adoptive immunotherapy using activated macrophages as immunotherapeutic agents.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $50,000

CONTRACT RESEARCH SUMMARY

Title: Effect of Transfer Factor on Virus-Induced Tumors in Marmoset Monkeys

Principal Investigator: Dr. Peter Baram
Name/Address Rush-Presbyterian-St. Luke's Medical
Performing Organization: Center
Chicago, IL

Contract Number: NO1-CB-43951
Starting Date: 6/28/75 Expiration Date: 12/27/76

Goal: This program was designed to investigate the effectiveness of tumor antigen-specific dialyzable transfer factor (TF_d) as an immunotherapeutic agent in the treatment of tumors.

Approach: Tumors are induced by the injection of autologous fibroblasts, transformed in vitro with the Schmidt Ruppin strain of Rous sarcoma virus (SR-RSV), into marmoset monkeys. The effect of TF_d (prepared from the lymphocytes of rhesus monkeys sensitized to autologous RSV transformed fibroblasts) therapy on the tumor is to be studied.

Progress: Fibroblast cell lines were established from rhesus and marmoset monkeys, and transformed with SR-RSV. Rhesus monkeys injected with autologous RSV transformed fibroblasts did not develop sensitivity to tumor antigen.

Marmosets were tested at four intervals for hypersensitivity to KLH and PPD by lymphocyte transformation and MIF production. Animals found to be negative to both received either KLH and PPD-TF_d or PPD-TF_d. Groups of 6 marmosets received 3 injections of TF_d. Sensitivity to KLH and PPD in the recipient animals was apparent one week following the first KLH and PPD-TF_d injection and increased with additional injection of TF_d. Recipient of PPD-TF_d also developed sensitivity to KLH at a lower level.

TF_d specifically recruits lymphocytes to antigens to which the donor is sensitive. It appears that the injection of TF_d also caused the appearance of a clone(s) of lymphocytes in the peripheral circulation, which are recruitable by antigens to which the host had no previous exposure.

Significance to Cancer Research: TF_d may be used to increase the number of recruitable lymphocytes, so that patients may develop and maintain CMI to tumor antigens.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 4/29/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Role of Circulating Tumor Antigens

Principal Investigator: Dr. Joseph D. Feldman
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: NO1-CB-43874
Starting Date: 3/1/75 Expiration Date: 2/28/78

Goal: To evaluate the effects of immunologic agents and reagents such as antigens, antigen-antibody complexes, and effector cells on the treatment of a virus-induced rat tumor.

Approach: Attempts will be made to augment and exaggerate the host immunologic responses that inhibit or destroy tumor in vivo and to suppress those responses that enhance tumor growth. This entails preparation of (a) membrane antigens of tumor cells; (b) cytotoxic antibodies of a single immunoglobulin class and specificity; and (c) purified populations of cytotoxic effector cells.

Progress: BN sarcoma (MST), induced by Moloney sarcoma virus and expressing virus on its cell surface, was fatal in BN hosts at a subcutaneous dose of 5×10^6 MST, and was rejected at a dose 0.5×10^6 MST. A unique protein containing tumor-specific antigens without detectable viral, fetal, or histocompatible antigens has been extracted from cell surface membranes and cytoplasm of MST. Studies have been undertaken to determine the appearance, persistence, and specificity of antibody to MST and to elucidate the tumor-enhancing or inhibiting qualities of the various IgG subclasses. The amount of antigen-antibody complexes detected in the sera of tumor-bearing rats depended on the size of the initial inoculum and size of tumor growth and disappeared when tumor was eliminated. Studies have been undertaken to determine the antitumor activity of cytotoxic T lymphocytes and peritoneal and splenic macrophages.

Significance to Cancer Research: It is essential to understand the complex interactions of the components of the immune system and to determine how best to manipulate these interactions to achieve a therapeutic benefit in cancer treatment.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $130,588

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Response

Principal Investigator: Dr. Yashar Hirshaut
Name/Address Sloan-Kettering Institute for Cancer
Performing Organization: Research
New York, NY

Contract Number: NO1-CB-53970
Starting Date: 5/16/75 Expiration Date: 5/15/78

Goal: To evaluate selected immunotherapeutic agents for acute and chronic toxicity, maximum tolerated dose, and effects on immune status. The two agents presently being investigated are Corynebacterium parvum (C. parvum) and levamisole.

Approach: Clinical protocols have been designed which provide for the administration of new immunotherapeutic agents to patients in accordance with acute, chronic and escalating dose schedules. Where feasible, the routes of administration include oral, parenteral and intralesional. During treatment toxic and immune parameters are measured at regular intervals.

Progress: In the first 18 months groundwork has been laid for conducting effective Phase I studies of immunomodulators. Means have been developed for assuring an adequate rate of patient entry, close monitoring of clinical and laboratory findings, supervision of drug administration, reliable abstraction of records, and the use of computers for data inventory and analysis. Over 130 patients have been entered in the C. parvum study which includes acute, escalating dose (I.V.), escalating dose (I.L.), chronic, and high dose (240-hour infusion) trials. There are 35 patients in the acute and chronic Phase I levamisole trials. The dose range for C. parvum used was 0.002 to 175.0 mg/m^2. The levamisole dose range was 75-625 mg/m^2. Common clinical toxicities for C. parvum I.V. and I.L. are fever, shaking chills, tachycardia, and hypertension. Levamisole toxicity is related to the gastrointestinal and nervous systems. Low dose I.V. C. parvum increases granulocyte function while decreasing lymphocyte response to specific antigens. With high doses there is a general depression of host immunity. Levamisole has as yet shown no consistent immune effects.

Significance to Cancer Research: Immunotherapy is a new modality of treatment for cancer whose appropriate use depends on understanding of how available agents affect specific aspects of immune function. Phase I studies underway should assist in determining optimal doses producing acceptable toxicity.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $147,015

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Immunotherapy with Tumor Preparation in Man

Principal Investigator: Dr. Herbert F. Oettgen
Name/Address Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, NY

Contract Number: NO1-CB-64062
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To determine the effectiveness of treating patients with advanced malignant melanoma with a combination of surgery, BCG, autologous tumor cell vaccine, and chemotherapy (DTIC). To monitor the patients' immune response to surface antigens of autologous and allogeneic melanoma cells, as measured by serological and cellular reactions and to correlate the response with the course of the disease and the effects of therapy.

Approach: Patients with malignant melanoma Stage III will be treated with BCG and a vaccine of autologous irradiated tumor cells. Patients with malignant melanoma Stage IV will receive BCG, irradiated autologous tumor cells, and DTIC. The patients' immune response to surface antigens of their own tumor cells will be tested in vitro by serological tests (mixed hemadsorption, immune adherence, complement-dependent cytotoxicity, antibody-directed cell-mediated cytotoxicity), and tests for lymphocyte cytotoxicity on autologous or allogeneic target cells.

Progress: From 1 July 1976 to 15 January 1977, 6 patients with malignant melanoma Stage III and 8 patients with malignant melanoma Stage IV were treated with autologous tumor cell (ATC) vaccine and BCG, alone (Stage III) or combined with DTIC (Stage IV). Serum from these patients was tested for antibodies to surface antigens of autologous cultured melanoma cells by mixed hemadsorption (MHA) and immune adherence (IA). Antibodies were detected in the serum of 4 patients by MHA, and with serum of 9 patients by IA. The specificity of antibodies in high-titered sera was defined by extensive absorption tests. Peripheral blood lymphocytes were tested for cell-mediated cytotoxicity (CMC) for autologous cultured melanoma cells prelabeled with ^{3}H-proline. CMC for autologous melanoma cells was detected in 9 of 13 patients. Correlation of the results of serological tests and lymphocyte cytotoxicity tests with vaccination and course of disease is underway. Therapeutic effects of vaccination cannot yet be evaluated.

Significance to Cancer Research: Malignant melanoma offers unique opportunities for study in that the serology is further advanced than in other types of cancer. Thus a trial of autologous vaccine will provide information concerning therapy as well as basic human cancer immunology.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Therapy of Tumors in Mice with Tumor Necrosis Factor (TNF)

Principal Investigator: Dr. Lloyd Old
Name/Address Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, NY

Contract Number: NO1-CB-64051
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To determine the effectiveness of tumor necrosis factor (TNF) in the therapy of transplantable and spontaneous mouse tumors.

Approach: TNF is found in the serum of BCG or C. parvum primed mice injected with endotoxin. Both TNF^+ serum and partially purified TNF are being tested for antitumor activity in the following five mouse tumor systems: Meth A sarcoma (BALB/C); leukemia L1210 (DBA/2); sarcoma 19B-MCA (C57BL/6); AKR spontaneous leukemia; and C3H spontaneous mammary carcinoma. Tumor necrosis, tumor growth, inhibition of metastases, and survival are the parameters to be assessed.

Progress: The two notable features of TNF are (a) its ability to cause acute necrosis of a variety of mouse tumors growing intradermally or subcutaneously and (b) its cytostatic and cytotoxic activity on certain lines of cultured cancer cells. Standard methods for its production and partial purification are now defined. During the contract period, a large pool of TNF serum has been prepared for (a) antitumor assays and (b) production of partially purified TNF. Single injections of TNF^+ serum cause acute necrosis of established Meth A and 19B-MCA solid tumors and significant reduction in the size of leukemic lymph nodes and spleen in AKR mice. In the Meth A and 19B-MCA systems, total tumor regressions have been observed following injection of TNF^+ serum and various parameters are now being explored to maximize this effect. Initial experiments have also indicated that infusion of TNF^+ serum causes some prolongation in the life of AKR leukemic mice.

Significance to Cancer Research: Immunological approaches to cancer therapy are being tested in laboratories and clinics around the world. Agents such as BCG and C. parvum, which augment immunological reactivity in a general fashion under certain circumstances and have tumor inhibitory activity in experimental systems, are receiving greatest attention in clinical trials of cancer immunotherapy. The past work leads to speculation that TNF may be one of the mediators responsible for the antitumor activity of these microbial immunopotentiators. Understanding how TNF causes tumor cell destruction and determining how effective it is as an antitumor agent may provide novel approaches to cancer immunotherapy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. parvum in Disseminated Carcinoma of the Breast

Principal Investigator: Dr. Carl Pinsky
Name/Address Sloan-Kettering Institute for Cancer
Performing Organization: Research
New York, NY

Contract Number: NO1-CB-53873
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To determine whether the addition of Corynebacterium parvum to combination chemotherapy in patients with advanced breast cancer increases response rate, increases response duration, increases survival time, protects against the deleterious effects of chemotherapy or augments immunologic function.

Approach: This is a prospective, randomized trial using cyclophosphamide, adriamycin, methotrexate and 5-fluorouracil every 28 days, with or without C. parvum given weekly, subcutaneously.

Progress: To date, 87 patients have been entered into the study (28 during this contract year). Despite the relatively small number of evaluable patients, it is already clear that there is no difference in the response rate and probably response duration between the group receiving chemotherapy alone, and the group receiving chemoimmunotherapy. On the other hand, the survival of responders who receive chemoimmunotherapy appears to be longer than survival of responders who receive chemotherapy alone.

Significance to Cancer Research: Since complete response rate is minimal and ultimate survival continues to be short in patients with advanced breast cancer, any lead which suggests that survival can be lengthened is noteworthy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Levamisole as a Therapeutic Adjunct in Squamous Cell Carcinoma of Head and Neck

Principal Investigator: Dr. Harold J. Wanebo
Name/Address: Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, NY

Contract Number: NO1-CB-53875
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To evaluate the usefulness of levamisole in the treatment of patients with squamous cell cancer of the head and neck.

Approach: Patients, stratified according to type of disease (primary or recurrent), stage and site, are randomized after surgery on a double blind basis to receive levamisole or a placebo. Treatment is given three times on alternate weeks for two years. The effects of levamisole on clinical course as well as on the immune status are being evaluated.

Progress: Of the 58 patients entered on study by January 1, 1977, 31 patients were randomized to levamisole; 27, to placebo. There were no statistically significant differences in the overall recurrence rates or remission duration between the two treatment groups. However, there were suggestive differences favoring levamisole in the recurrence rate of those patients operated upon for primary cancer. Immunological testing demonstrated that DNCB responses and lymphocyte responses to mitogens were improved in patients after tumor resection prior to beginning the levamisole-placebo protocol. Levamisole treatment appears to augment the lymphocyte count, but does not change lymphocyte responses to mitogens. Lymphocyte counts and mitogen responsiveness appear to be decreased in patients who develop recurrences.

Significance to Cancer Research: Immunopotentiation is a promising clinical approach to cancer therapy especially when used as an adjuvant in patients with known immunosuppression. Levamisole, an immune modulator, has been reported to be useful in the treatment of resectable lung cancer and in patients treated for locally advanced breast cancer with radiation (Rojas et al.). This study will attempt to determine whether levamisole has therapeutic efficacy in patients with head and neck cancer.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Herpes Virus Lymphomas in Marmosets

Principal Investigator: Dr. Jorg W. Eichberg
Name/Address Performing Organization: Southwest Foundation for Research and Education
San Antonio, TX

Contract Number: NO1-CB-64055
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: Immunotherapy of cancer.

Approach: The use of specific transfer factor and immune RNA, and a combined regimen with both of these components to immunotherapeutically treat fatal lymphoproliferative malignancy induced by Herpesvirus saimiri in marmosets.

Progress: Adult baboons were immunized with Herpesvirus saimiri (HVS) to serve as a source of transfer factor (TF) and immune RNA (IRNA). All donors responded with positive lymphocyte blastogenic responses to HVS when their peripheral blood lymphocytes (PBL) were assayed. However, at sacrifice, when aliquots of lymph node lymphocytes (LNL) and TF-lymphocytes (from combined LNL + PBL) were assayed, the response was generally negative, possibly demonstrating suppressor cell activity.

Positive transfer of cellular reactivity to HVS in vivo to marmoset recipients by TF could be demonstrated at one time or another by either lymphocyte blastogenesis or migration inhibition assay. T-rosette formation or cytotoxic lymphocytes were negative. Marmosets which received TF are currently challenged with HVS. IRNA has been extracted from spleens of immunized baboon donors and is currently evaluated for efficacy of transfer of cellular reactivity.

Significance to Cancer Research: HVS-induced lymphoproliferative disease is absolutely reproducible in cotton-top marmosets and clinically similar to human cancer. This animal model provides a direct phylogenetic relationship to man, and offers the opportunity to evaluate immune reactivity and therapy between species by TF and/or IRNA.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy with In Vitro Lymphocyte Sensitization

Principal Investigator: Dr. Henry S. Kaplan
Name/Address Stanford University
Performing Organization: Stanford, CA

Contract Number: NO1-CB-63988
Starting Date: 9/1/75 Expiration Date: 2/28/78

Goal: (1) Development of in vitro methods for sensitization of human and mice lymphocytes against allogeneic and syngeneic tumor cells. (2) Analysis of the cell populations involved in such sensitization and of the regulatory mechanisms that control the induction and effector phases of the response. (3) Inhibition of tumor development by adoptive transfer of the in vitro sensitized lymphocytes.

Approach: Mouse spleen cells or human peripheral blood lymphocytes are sensitized in vitro against tumor cells either by direct interaction with irradiated tumor cells or by exposure to syngeneic macrophages which were fed with cell-free extracts of tumor cells. A mouse lymphoma model is used to test sensitized lymphocytes for their activity against target cells in vitro, and for their influence on tumor development in vivo.

Progress: Primary induction of cell-mediated immune response in vitro against tumor antigens was achieved in animal models by (1) incubation of lymphocytes with irradiated tumor cells; (2) presenting of cell-bound antigens to lymphocytes by syngeneic macrophages. The second was adapted to human tissues by using uninvolved spleens from Hodgkin's and lymphoma patients as a source for macrophages and lymphocytes. We found that human lymphocytes could be sensitized against tumor cells by antigen-fed macrophages. However, lymphocytes sensitized by macrophages manifested cytostatic, but not cytotoxic, activity against tumor cells. In parallel, we developed a method of induction of cytotoxic lymphocytes against human lymphoma cell lines, by direct sensitization against irradiated lymphoma cells of T-cell enriched population of peripheral blood lymphocytes. The radiation-induced leukemia virus in mice was used for analysis of the macrophage-mediated sensitization and for studying the in vivo activity of in vitro sensitized lymphocytes. Peritoneal macrophages which had been fed with viral preparations could sensitize syngeneic lymphocytes against viral-associated antigens. The sensitized lymphocytes, although only cytostatic in vitro, could inhibit lymphoma development in vivo.

Significance to Cancer Research: The in vitro modification of the cellular immune response against tumor antigens is of primary importance for understanding the natural immune response of the host against malignant cells and for directing such a response to inhibit tumor development.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $126,509 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Modified Tumor Cell Membranes as Immunotherapeutic Agents

Principal Investigator: Dr. Jack H. Pincus
Name/Address Stanford Research Institute
Performing Organization: Menlo Park, CA

Contract Number: NO1-CB-64053
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To evaluate the effect of glycosidic modifications of plasma membranes and membrane glycoproteins on their capacity to induce an immunological regression of growing tumors. To determine which of these glycosidic modifications optimizes the immunotherapeutic effectiveness of these cell-free preparations.

Approach: Purified plasma membranes are prepared from the L1210 tumor, a transplantable leukemia that is propagated in DBA/2 mice. The purified plasma membranes are treated with purified glycosidic enzymes to remove sequentially specific carbohydrates from their oligosaccharide side chains. The modified plasma membranes are tested for their effectiveness in inducing an immunological regression of growing L1210 tumors.

Progress: Methods were developed for removing erythrocytes from L1210 ascites tumor cells and plasma membranes were prepared from the erythrocyte-free tumor cells. Conditions were developed for quantitative removal of sialic acid from plasma membrane oligosaccharides with Vibrio cholerae neuraminidase. Optimal conditions also were developed for quantitative removal of galactosyl residues by -galactosidase. An ascites tumor model was chosen to evaluate the immunotherapeutic effectiveness of neuraminidase-treated plasma membranes in DBA/2 mice bearing growing L1210 tumors. Mice injected intraperitoneally with neuraminidase-treated plasma membranes five days after tumor cell inoculation survived significantly longer than untreated mice. Untreated plasma membranes were less effective in prolonging survival than neuraminidase-treated plasma membranes. A small percentage (5%) of tumor-bearing mice receiving neuraminidase-treated plasma membranes appeared to have rejected their tumors.

Significance to Cancer Research: Defining those glycosidic modifications of tumor cell membranes that increase their immunogenicity will aid in developing vaccines for use in the active immunotherapy of small tumors of man or tumors remaining after surgical removal of the primary tumor.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Study of Microorganisms as Inhibitors of Tumor Cell Growth

Principal Investigator: Dr. Frank M. Collins
Name/Address Trudeau Institute, Inc.
Performing Organization: Saranac Lake, NY

Contract Number: NO1-CB-23221
Starting Date: 2/1/76 Expiration Date: 1/31/77

Goal: To compare different Mycobacteria for their ability to grow and survive within the lymph nodes and spleen of guinea pigs subject to various intradermal inocula and to examine their ability to augment the development of specific antitumor immunity.

Approach: To compare the cellular responses in mice and guinea pigs exposed to different BCG preparations and to select the best strain which will then be introduced by skin scarification into normal and tumor-bearing guinea pigs and the growth and spread of both BCG and tumors in the draining lymph nodes will be followed quantitatively.

Progress: A study was made of the lymphatic drainage of normal strain 2 guinea pigs using the vital dye Chicago Blue injected intracutaneously into flank skin, subcutaneously into the footpad, or intramuscularly in the leg. The passage of dye through the lymphatics was compared with that seen when viable BCG Pasteur was injected into the same sites. The pattern of lymphatic drainage was established by serial viable counts on individual lymph nodes.

The effect of dose and the number of multiple scarification sites on spleen and lymph node counts was established and the effect of repeated reinfection with viable BCG on the growth patterns in vivo were compared in normal and tuberculin hypersensitive animals.

The effect of cortisone treatment on tuberculin hypersensitivity and in vivo growth patterns for intracutaneous inocula of BCG Pasteur in presensitized and normal guinea pigs was compared quantitatively with a view towards establishing the effect of immunosuppression on the growth and spread of BCG in the lymph nodes of intracutaneously infected animals.

Significance to Cancer Research: BCG has been used largely empirically in the immunotherapy of cancer. These studies provide quantitative data on the growth of BCG in normal and tuberculin hypersensitive animals which can be used to better predict the likely behavior of BCG in tumor-bearing animals.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: The Production of an Improved BCG Vaccine

Principal Investigator: Dr. Thomas Miller
Name/Address Trudeau Institute, Inc.
Performing Organization: Saranac Lake, NY

Contract Number: NO1-CB-53885
Starting Date: 8/23/74 Expiration Date: 8/22/77

Goal: To prepare, for use in man, a fresh frozen preparation of BCG vaccine (BCG-FF), which (1) would have immunopotentiating characteristics equal or superior to the commercially available products and (2) would have the added advantage of not containing the undesirable side effects of vaccines containing dead and disrupted bacilli.

Approach: The BCG-FF was grown in submerged culture in completely synthetic (nonantigenic) medium with careful attention given to conditions which would provide a high density of growth in which virtually every bacillus would be intact and living.

Progress: A vaccine of high viability was produced in a medium free of extraneous allergens. The yield of vaccine was greater than 1×10^9 viable bacilli per ml; this was achieved by optimizing the concentration of wetting agent, changing the source of carbon from dextrose to glycerol and increasing the aerobic potential of the culture medium by using a tissue culture roller apparatus. Ten thousand vials of this vaccine were supplied to the National Cancer Institute for an evaluation of its clinical efficacy in the immunotherapy of cancer.

Significance to Cancer Research: Experiments in animals showed that the BCG-FF possessed greater immunomodulating potency and immunotherapeutic activity than other vaccines. In addition, the BCG-FF did not possess immediate dermal necrotic activity following intradermal injection.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Specific and Nonspecific Immunotherapy as an Adjuvant to Chemotherapy

Principal Investigator: Dr. Frederick Eilber
Name/Address
Performing Organization: University of California
Los Angeles, CA

Contract Number: NO1-CB-53941
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To determine if post-surgical adjuvant chemoimmunotherapy is superior to adjuvant chemotherapy in preventing disease recurrence in patients with highly malignant skeletal or soft-tissue sarcomas.

Approach: Patients with osteosarcoma, rhabdomyosarcoma, synovial cell or anaplastic sarcoma following complete surgical excision of their tumor are randomized between chemotherapy -- adriamycin, high-dose methotrexate and citrovorum rescue -- and identical chemotherapy plus immunotherapy consisting of BCG and allogeneic tissue culture sarcoma tumor cell vaccine. Chemotherapy is given for one year, the immunotherapy is given for the identical year plus an additional year.

Progress: Using conventional therapy all patients entering this study have less than a 20% chance of remaining disease free for two years. To date, 40 patients are on study. Twenty have received chemotherapy alone and 20 have received adjuvant chemoimmunotherapy. Preliminary results with a median follow-up of 14 months indicate that 78% of the patients remain disease free. Because so few patients have thus far recurred, no statement can be made as to the efficacy of chemo- versus chemoimmunotherapy.

Significance to Cancer Research: The significance of this project has been to reduce disease recurrence and to prolong survival in a group of diseases that was previously rapidly fatal. Since the chemotherapy is discontinued after one year, it will be of major importance to determine if those patients who continue to receive immunotherapy will have a more lasting disease-free status than those patients receiving the chemotherapy alone.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $160,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. parvum in Advanced Cancer of the Breast and Lung

Principal Investigator: Dr. John Fahey
Name/Address
Performing Organization: University of California
Los Angeles, CA

Contract Number: NO1-CB-53939
Starting Date: 6/16/75 Expiration Date: 6/15/78

Goal: To evaluate the usefulness of C. parvum and/or BCG as adjuncts to chemotherapy in the treatment of disseminated breast cancer and lung cancer.

Approach: Patients with breast cancer who have received no previous chemotherapy other than hormonal agents will continue to be randomized to treatment with adriamycin and cyclophosphamide with or without weekly subcutaneous C. parvum (protocol BP-2). Patients with breast cancer who have received prior chemotherapy will be randomized to adriamycin and vincristine with or without weekly subcutaneous C. parvum (protocol BP-3). In both instances, patients not receiving C. parvum will be receiving a sterile water placebo. Lung cancer patients will be randomly assigned to chemotherapy (cytoxan, methotrexate, adriamycin and vincristine) with or without BCG (protocol LP-1).

Progress: Ten patients have been entered on protocol BP-2. Protocol BP-3 is just being activated. In the past 12 months, 60 patients entered the lung cancer protocol LP-1 and were randomly assigned to chemotherapy alone, chemotherapy plus BCG (well tolerated), or chemotherapy plus intravenous C. parvum (poorly tolerated). Because of the significant toxicity encountered with i.v. C. parvum, this treatment arm is to be discontinued. Thus far, immunotherapy did not produce a significant difference in response rate, time to progression, or hematopoietic tolerance to chemotherapy.

Significance to Cancer Research: Cancer of the breast and lung are two of the most common varieties of cancer. Evaluation of immunotherapy as an adjunct to chemotherapy in these tumors has a high priority.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $105,000

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy

Principal Investigator: Dr. Donald L. Morton
Name/Address University of California
Performing Organization: Los Angeles, CA

Contract Number: NO1-CB-64076
Starting Date: 8/8/74 Expiration Date: 8/7/78

Goal: To determine whether immunotherapy with BCG alone or BCG combined with tumor cell vaccine will decrease recurrence rate or prolong survival in melanoma patients with metastases to regional lymph nodes.

Approach: Patients are randomized following surgical lymphadenectomy into 3 groups receiving different postoperative therapy: one group receives no additional therapy, one receives BCG, and one group receives BCG and allogeneic tumor cell vaccine.

Progress: During the past year 38 patients have been entered in the study (in addition to the 85 evaluable patients entered previously). It now appears that patients receiving immunotherapy are surviving significantly longer than control patients. These overall differences are maintained when corrections are made for all prognostic factors, except Clark's levels. When corrections are made for Clark's levels, the data do not quite achieve a $p<0.05$ level of significance.

Significance to Cancer Research: If further patient accrual and analysis substantiate the trend toward a positive benefit of immunotherapy, a significant advance will have been made in the treatment of patients with malignant melanoma metastatic to regional nodes.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 3/10/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $332,723 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Immune Status of Patients and Effects of Immunostimulants on Radiation Therapy

Principal Investigator: Dr. William M. Wara
Name/Address University of California
Performing Organization: San Francisco, CA

Contract Number: NO1-CB-64004
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To document the immunosuppression which occurs following radiation therapy and to determine if a therapeutic advantage can be achieved by the addition of immunotherapy (thymosin) to the conventional treatment given for squamous cell carcinoma of the head and neck region and the esophagus.

Approach: Both groups of head and neck and esophageal cancer patients will receive radiation therapy and/or surgery as treatment. Equal numbers of comparable patients will then be randomized to receive thymosin (Hoffmann-La Roche) or placebo in addition to their usual standard treatment. All patients will be monitored with immunologic tests to measure antibody-mediated and cell-mediated immunity at regular intervals. At the completion of the study the data will be analyzed for local disease recurrence, metastatic disease, and survival difference in the patient groups.

Progress: Over 70 controls have been evaluated and their data accumulated to ascertain quality control of our laboratory results. Twenty-two patients have been studied in the first quarter of the contract and their early results indicate the expected immunosuppression after irradiation. Hoffmann-La Roche, the supplier for thymosin and placebo, has now been able to supply adequate amounts of biologically active material so that the randomized trial may begin.

Significance to Cancer Research: The results of this study will document prospectively the significance of immunosuppression caused by irradiation in head and neck and esophageal cancer patients and determine whether the effect of immunotherapy (thymosin) in reversing this immunosuppression has any impact on the disease-free interval, survival, and local control of cancer in these patients.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $92,000

CONTRACT RESEARCH SUMMARY

Title: Active Specific Immunotherapy in Acuté Myelogenous Leukemia

Principal Investigator: Dr. Jacob Zighelboim
Name/Address University of California
Performing Organization: Los Angeles, CA

Contract Number: NO1-CB-64006
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To evaluate the effects of immunizing patients with acute myelogenous leukemia or acute myelomonocytic leukemia with modified allogeneic leukemia blast cells.

Approach: Eligible patients who are in bone marrow remission and receiving consolidation or maintenance chemotherapy will be randomized into three groups according to the dosage and schedule of immunizing cells used. The study will be performed in two sequential phases. Patients randomized during Phase A will receive immunization with lethally irradiated leukemia cells, and those randomized during Phase B will receive vibrio-cholera-neuraminidase-treated leukemia cells.

Progress: As of April 1, 1977, 4 patients have received injection of allogeneic irradiated cells without untoward complications. Immunological tests reveal (a) higher stimulation of DBA synthesis by the immunizing cells in 2 patients, while in one patient no difference was seen; (b) increase in antibody titer in 2/3 patients after 304 immunizations; and (c) direct cell-mediated cytotoxicity was not detected in any patient. After 48-72 hours of in vitro culture with the immunizing cells, cytotoxic lymphocytes were generated which were able to induce cytolysis of the immunizing allogeneic myeloblasts and some B-lymphoblastoid cell lines, while they did not react with T-lymphoblastoid cell lines, peripheral blood lymphocytes, and most of the tested allogeneic leukemic cells. Maintenance chemotherapy with cytosine arabinoside and G-thioguanine produced a remarkable leukopenia. A significant decrease in circulating T lymphocytes, B lymphocytes, K cells, and null cells was detected as early as 14 days after a course of chemotherapy and usually between the third and fourth week. These effects usually lasted for 4-5 weeks and sometimes longer and were associated with a marked decrease in antibody-dependent cellular cytotoxicity (ADCC) and lectin-dependent cellular cytotoxicity (LDCC).

Significance to Cancer Research: It is important to assess the value of active specific immunotherapy in the treatment of human cancer and to explore in depth the interaction of immunotherapy with immunosuppressive chemotherapy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: $96,000

CONTRACT RESEARCH SUMMARY

Title: Assessment of Tumor-Specific Immunity in Osteosarcoma Patients

Principal Investigator: Dr. Richard T. Smith
Name/Address University of Florida
Performing Organization: College of Medicine
Gainesville, FL

Contract Number: NO1-CB-53933
Starting Date: 6/16/75 Expiration Date: 6/15/77

Goal: The goal of this contract year was to develop methods of detecting specific lymphoid cell-mediated and antibody-mediated reactions to tumor antigens and to apply these tests to patients with osteosarcoma, other neoplastic diseases, or benign bone disorders; normal blood donors; and family members of osteosarcoma patients.

Approach: Cell-mediated immunity to osteosarcoma was tested using a lymphocyte proliferation assay (LPA) to 3M KCl solubilized osteosarcoma antigen. Antibody-mediated immunity was measured by either of two methods: passive hemagglutination in which solubilized osteosarcoma antigen is covalently bonded to sheep erythrocytes, which may be agglutinated by antiserum; or immunofluorescence on frozen tissue sections.

Progress: To date, 358 different persons have been assayed by LPA. Seventy-five percent of tumor-bearing patients are reactive in this assay, while only 40% of tumor-free patients are reactive. Some patients' family members (45%) are reactive, but less than 20% of nonosteosarcoma patients or normal individuals are reactive. Over 200 sera have been tested by passive hemagglutination for antibody to osteosarcoma antigen. Sera from the tumor-bearing patients were negative while about 50% of tumor-free sera were positive. All normal sera were negative, but 50% of patient family members were positive. An immunofluorescence (IF) assay was developed. Only a small number of sera have been tested by IF to date, but one serum obtained from a family member has shown specificity for osteosarcoma.

Significance to Cancer Research: These methods may be effective in assessing the tumor-bearing status of osteosarcoma patients; LPA reactivity with no antibody titer being consistent with tumor-bearing and high antibody titer with low LPA reactivity being indicative of tumor-free status. The IF assay has probable diagnostic value.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Human Lung Cancer -- Evaluation of BCG Therapy

Principal Investigator: Dr. Robert H. Waldman
Name/Address University of Florida
Performing Organization: Gainesville, FL

Contract Number: NO1-CB-43857
Starting Date: 11/15/74 Expiration Date: 11/14/76

Goal: Evaluation of the effectiveness of aerosolized (inhaled) BCG plus standard surgery and chemotherapy, as compared to standard therapy alone, in treatment of bronchogenic carcinoma.

Approach: A randomized trial was undertaken in which patients were placed in either a placebo or BCG group. This therapy was in addition to the usual treatment the patients received for bronchogenic carcinoma. The saline placebo or BCG was administered by aerosol.

Progress: At the present time, 21 patients have been admitted to the study, nine in the BCG group, and 12 in the placebo group. Survival curves indicated that the 50% survival time in the saline group is 8.5 months. In the BCG group, at the present time, we have seen a 68% survival. Thus, it would appear that the patients in the BCG group have an improved survival as compared to the placebo group.

Significance to Cancer Research: If these preliminary results are confirmed with a greater number of patients and for a longer period of time, it would appear that aerosolized BCG improves survival in patients with lung cancer.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Date: 5/18/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Role of Circulating Tumor Antigens

Principal Investigator: Dr. Charles F. McKhann
Name/Address University of Minnesota
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-43946
Starting Date: 5/1/74 Expiration Date: 4/30/77

Goal: To evaluate the kinetics of production of tumor antigen and antigen-antibody complexes in the circulation of tumor-bearing mice, comparing mice that received immunosuppressive irradiation with those that did not. Circulating antigen and antigen-antibody complexes were to be evaluated in relation to tumor size and lymphocyte refractory state. Organ deposition of antigen-antibody complexes were to be studied. Tumor antigen and antigen-antibody complexes were to be studied for stimulation of lymphocytes.

Approach: Antigens were extracted from murine methylcholanthrene-induced sarcomas by the 3M KCl technique of Reisfeld and Kahan. The Raji cell radioimmunoassay was evaluated for its ability to measure complexes in the serum of mice. Stimulation of immune lymphocytes was used to monitor the presence of antigen in serum and extracts. Fluorescent antibody was used to detect complexes in the kidneys of normal mice and in mice bearing tumors.

Progress: The major finding of this study thus far is that antigen recovered from tumor cells could be used to stimulate lymphocytes in vitro. The antigens appear to be heterogeneous, may be lipoproteins, are not fetal antigens, and do not contain IgG. Complexes: The Raji radioimmunoassay reveals no statistically significant difference in levels of circulating complexes between mice bearing fibrosarcomas and their normal age matched counterparts. However, in C3H/HeJ female mice the level of whatever substances in the serum the assay measures increases from 3 to 12 weeks and then levels off. Fluorescence microscopy reveals that both normal and tumor-bearing C3H/HeJ female mice have deposits containing C3 and IgG in their kidneys and that the amounts of these deposits increase with age.

Significance to Cancer Research: Kinetics of circulating tumor antigens may have a profound effect on proper design of courses of immunotherapy; the recovery of antigen-antibody complexes from the serum of tumor-bearing individuals will provide an excellent source of highly purified antigen and antibody when split. The use of soluble antigen as an immunogen in immunotherapy will require further study to determine the role of such material in lymphocyte stimulation and cellular cytotoxicity.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee of Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in Immune Response to Tumors

Principal Investigator: Drs. Jon R. Schmidtke and Robert Nelson
Name/Address University of Minnesota
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-43948
Starting Date: 6/1/74 Expiration Date: 5/31/78

Goal: To study the role of macrophage function in the afferent and efferent phases of immune response in both murine and human systems. In murine systems: to determine a number of macrophage functions in both normal and tumor-bearing animals as a function of time after introduction of a syngeneic tumor, and to determine if there are changes in macrophage function with progressive tumor growth which may affect the ability of these cells to regulate the immune response to tumors. In human systems: to develop six in vitro quantitative assays of macrophage function and to apply them to monocytes taken from both normal and tumor-bearing individuals.

Approach: To study macrophage function using two syngeneic murine tumors. Assays were developed to determine the ability of macrophages to phagocytose antigen, to measure metabolism of tumor and other antigens, and the ability of macrophages to transfer immunogenicity to protein antigens. These assays are done as a function of time after introduction of tumor into syngeneic hosts. In addition, cytochemical and functional assays were developed which selectively measure monocyte function in a mixed population of cells derived from peripheral blood.

Progress: During the past three years it was determined that macrophages from tumor-bearing mice do not differ significantly from normal macrophages in their ability to take up, catabolize and transfer immunogenicity to protein antigens. In addition, it was shown that macrophages taken from adjuvant treated donor mice, when transferred to syngeneic tumor-bearing mice, have significant immunotherapeutic potential in tumor-bearing hosts.

In the studies of human monocyte functions six separate functional tests for monocytes were developed. After studies evolved to enumerate monocytes, the contractor investigated lysosomal enzyme levels, chemoluminescence response, receptor function, migration of monocytes, and phagocytic function, and cell surface receptors.

Significance to Cancer Research: Data on macrophage function in murine systems show the immunotherapeutic potential of macrophages in humans when these cells are removed from an individual, activated in vitro with adjuvants and reinfused into tumor-bearing individuals. Assays are being developed to characterize and more accurately quantitate the monocytes involved in tumor rejection.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Cancer in Man

Principal Investigator: Dr. Richard L. Simmons
Name/Address University of Minnesota
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-23885
Starting Date: 2/1/75 Expiration Date: 4/30/77

Goal: Attempts to apply to patients with malignant melanoma (Stages II and III) an immunotherapeutic modality which has proven successful in causing the regression of solid tumors in mice. The technique involves removing the patient's tumor, treating with neuraminidase in vitro, and re-injecting the lethally irradiated tumor cells with BCG back into the patient.

Approach: The therapy was always combined with the optimal current therapy available, i.e., surgery for Stage II and chemotherapy for Stage III disease.

Progress: The contract has been completed. The results demonstrate no beneficial effect and no harmful effect of combining active specific immunotherapy with neuraminidase-treated cells along with standard modes of therapy for melanoma, Stages II and III.

Significance to Cancer Research: The failure to confirm in man with malignant disease the very promising results in animals may represent the failure of this particular disease to respond to this particular immunotherapeutic modality or, more likely, the inapplicability of the model system for this disease in man. The technique of active specific immunotherapy which would require nearly total destruction of the patient's own tumor followed by active specific immunotherapy seems not to be a promising one in man if autochtonous cells are required. If cultured cells or allogeneic cells could be utilized, the method would be more promising.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program Site Visit Dates: 12/5-6/74
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Study of Mycobacterium phlei in Lung Cancer, Hypernephroma and Malignant Melanoma

Principal Investigator: Dr. Gerald J. Vosika
Name/Address University of Minnesota
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-64002
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: (1) To establish the clinical dose, toxicity, and efficacy of intradermal and intralesional Mycobacterium phlei cell wall (CW), cell wall skeleton (CWS), and CWS plus P3 lipid; (2) to evaluate the effects and mechanism of action of M. phlei preparations as an immunostimulant and as a macrophage activator.

Approach: Patients with advanced lung cancer, hypernephroma, and malignant melanoma are entered into study. Patients receive either intradermal or intralesional inoculation of the vaccine weekly. The dose is escalated to establish a maximum tolerable level. Weekly tests of lymphocyte and monocyte cell populations and function are being carried out to determine the immunological effects of the vaccine and to correlate this with clinical response.

Progress: During the first six months of this contract, we have entered 16 patients onto our protocol. This represents 80% of all possible candidates. All patients have received the combined cell wall skeleton plus P3 lipid, either intradermally or intralesionally. Single intralesional doses up to 450 mg have been utilized without prohibitive toxicity. Weekly intralesional doses for 8 weeks utilizing 50 μg to 300 μg have been given. When utilized at different sites, no prohibitive toxicity was observed. Multiple injections of a single site eventually causes sterile abscesses. Intradermal inoculation has, on occasion, resulted in ulceration.

There have been 12 patients treated who had measurable disease. Two patients with malignant melanoma have shown a response, one complete and one partial. In the patient with a complete response, all disease (72 subcutaneous nodules) regressed completely after a period of three months after only 3 weekly injections of CWS of 300 μg each, or a total dose of 900 μg.

Significance to Cancer Research: The efficacy of a small amount of CWS to cause regression of disease in some patients makes this an extremely interesting material. Through the continued clinical trials and the thorough immunological study of these types of patients, it is possible to better understand and manipulate mechanisms of host defense.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Syngeneic Tumors in Wistar Rats

Principal Investigator: Dr. Robert W. Baldwin
Name/Address University of Nottingham
Performing Organization: Nottingham, England

Contract Number: NO1-CB-64042
Starting Date: 6/18/76 Expiration Date: 6/17/78

Goal: Evaluate the relative effectiveness of bacterial immunostimulants including BCG and C. parvum for active specific immunotherapy and adjuvant contact therapy of transplanted rat tumors especially those reviewed as being relevant models for human disease.

Approach: Two modalities of treatment are being evaluated in susceptible systems such as carcinogen-induced rat sarcomas to define optimum conditions. These procedures are then being used to determine their value in the treatment of more clinically relevant systems, e.g., transplants of spontaneous rat sarcomas and mammary carcinomas. In each system the effectiveness of therapy is being assessed against local tumor and metastatic deposits.

Progress: Adjuvant contact therapy is the most effective approach for treating the rat tumors used in this project. Both BCG and C. parvum are able to suppress growth of immunogenic and non-immunogenic tumors, including spontaneous mammary carcinomas and sarcomas which are viewed as relevant models for human cancer. The general concept that these bacterial preparations are most effective when contacted with tumor cells is emphasized by the finding that systemic administration of C. parvum did not influence subcutaneous tumors. In contrast, tumors developing at several sites including subcutaneous and pulmonary tissues and the pleural cavity were suppressed when C. parvum was administered directly to the tumor site. A screening system using rat sarcomas has been established to evaluate the effectiveness of bacterial preparations. BCG preparations show widely different activities, TMC 1011, Trudeau Pasteur and TM 1012 Trudeau Montreal being most effective.

Active specific immunotherapy using vaccines containing tumor cells and bacterial adjuvants can be used to control growth of transplanted tumors, but this approach is limited to those with well-defined tumor rejection antigens. The limitation of this type of therapy is indicated by its lack of effectiveness with spontaneously arising rat sarcomas and mammary carcinomas, which are more relevant than carcinogen-induced tumors for human disease.

Significance to Cancer Research: The studies of adjuvant contact therapy are relevant to the developments in clinical studies using intralesional local administration injection of bacterial adjuvants for therapy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Hapten Treatment of Cancer

Principal Investigator: Dr. Kazuhiko Arai
Name/Address University of Pennsylvania
Performing Organization: Philadelphia, PA

Contract Number: NO1-CB-43967
Starting Date: 5/16/74 Expiration Date: 5/15/77

Goal: To develop a new immunotherapy system, which utilizes a hapten and theoretically does not require cancer-specific antigen, into a clinically applicable methodology.

Approach: After a hapten-conjugated tumor protein is created in situ by direct injection of a hapten into the tumor, antiserum against the hapten-tumor conjugate and/or against the hapten is injected intravenously to evoke an Arthus-type reaction at the target tumor tissue.

Progress: The haptenic activity and direct conjugating ability of L-phenylalanine mustard (PhM) has been shown. A single treatment completely regresses an established allogeneic Walker tumor and a syngeneic fibrosarcoma (MC1) in LEW/Mai rat. Recent studies indicate: (1) the treatment is also effective against syngeneic rat adenocarcinoma (AC33); (2) repeated treatment is better than single treatment; (3) absorbed xenogeneic rabbit antiserum is effective without any side effects; (4) the single treatment of only one tumor in a rat with bilaterally similar transplanted two MC1 tumors caused complete regression of both the treated and untreated tumors, confirming previous results obtained with an allogeneic two-tumor model. Complete excision of tumor immediately after treatment significantly reduces the regressive effect on the remaining untreated tumor, indicating the importance of the presence of the treated tumor tissue for regression of the untreated tumor. Syngeneic antiserum produced by PhM-conjugated to noncancer carrier protein was equally effective. Data indicate that the tumor regression is not due to PhM alone, antiserum alone, nor by genetic factors.

Significance to Cancer Research: The results indicate that this approach to immunotherapy can regress an established tumor and its metastasis. The present data support the continued development of this mode of therapy for clinical application.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Immunity Induced by Drug (DTIC)-Mediated Tumor Antigens

Principal Investigator: Dr. Enzo Bonmassar
Name/Address University of Perugia
Performing Organization: Perugia, Italy

Contract Number: NO1-CB-64054
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: Studies concerning the origin and the properties of new tumor-associated transplantation antigens induced in cancer cells following treatment of tumor-bearing mice with drugs.

Approach: Experiments of *in vivo* transplantation resistance or *in vitro* cell-mediated cytotoxicity elicited by tumor cells antigenically altered by dimethyl-triazeno-carboxamide (DTIC).

Progress: Treatment of lymphoma-bearing mice with DTIC can induce new DTIC-mediated tumor antigens (DMTA). DMTA were found to be strong transplantation antigens, capable of eliciting T cell-mediated responses. In addition, DTIC-tumor sublines retained tumor-associated transplantation antigens (TATA) present in the tumor lines of origin. The results obtained during the present contract show that: (a) No cross reactivity could be found between DMTA and normal tissue antigens of DTIC-treated mice. (b) The kinetics of growth and regression of DTIC sublines in mice were similar to those of H-2-incompatible tumors. Transplantation resistance seems to be genetically regulated. (c) Secondary cytotoxic responses *in vitro* can be obtained by sensitization of lymphocytes with DTIC-lines: limited or no antigenic cross-reactivity was found among these sublines. (d) A nonimmunogenic $H\text{-}2^{b}$ lymphoma acquired strong DMTA following DTIC treatment for a number of transplant generations. (e) Response of Hh-type was found in irradiated mice against DMTA. One of the most relevant data seems to be the induction of strong DMTA in nonimmunogenic lymphoma cells (i.e., tumor cells lacking TATA recognized by syngeneic mice).

Significance to Cancer Research: The possibility of inducing new antigenic targets on cancer cells by treating the tumor-bearing host with drugs could open new perspectives in cancer immunotherapy.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: Approx. $67,000

CONTRACT RESEARCH SUMMARY

Title : Immunotherapy of C_3H Murine Mammary Carcinomas

Principal Investigator: Dr. Bernard Fisher
Name/Address University of Pittsburgh
Performing Organization: Pittsburgh, PA

Contract Number: NO1-CB-64044
Starting Date: 6/16/76 Expiration Date: 6/15/78

Goal: To improve inhibition of the growth of murine mammary carcinoma, both primary and metastatic, presently obtained by the combination of immunotherapy (C. parvum; CP) and chemotherapy (cyclophosphamide; CY) via the addition or substitution of various immunomodulators and chemotherapeutic agents, as well as by varying the route, sequence and other aspects of administration.

Approach: Other nonspecific stimulating agents such as BCG, tilorone, levamisole, Glucan and Bru-Pel, a nonviable insoluble residue of B. abortus, are being substituted for, or combined with C. parvum. The use of irradiated tumor cells as a specific immunostimulating agent is being explored as well as the use of subtumoricidal radiation of the primary tumor prior to institution of therapy. Both systemic and local application of the immunostimulating agents is being examined.

Progress: Tilorone, levamisole, BCG and MSF-MB, at various doses and schedules of administration, failed to inhibit tumor growth to the same extent as did C. parvum. This was observed when such agents were employed alone or in combination with CY. Levamisole and MSF-MB failed to do so. Tilorone or MSF-MB did not influence the effect on tumor growth when employed alone, with CP, CY or CP + CY. Experiments begun to determine whether local immunotherapy not only affects the treated tumor but distant foci as well indicated that CY with intra-tumor CP results in almost 100% total permanent regression, greatly superior to results with systemic CP. Administration of CP in an emulsion or as complete Freund's adjuvant failed to demonstrate an advantage. Radiation of tumors (2000 R), which had only slight effect on tumor growth, enhanced the effect of subsequently administered CP as well as CY + CP.

Significance to Cancer Research: For effective cancer management, systemic therapy must be utilized. Chemotherapy is unlikely to eliminate the total tumor cell population. By increasing the host immune response with immunostimulating agents, residual tumor cells may be eliminated. Successful treatment will result when all modalities can be utilized, together under optimal circumstances.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutic Studies with Lymphokine 1788

Principal Investigator: Dr. Ben W. Papermaster
Name/Address University of Texas Medical Branch
Performing Organization: Galveston, TX

Contract Number: NO1-CB-64058
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: Production and characterization of cultured lymphoid cell lymphokine fractions, which induce inflammation and regression of susceptible tumors in animals and man.

Approach: Cell culture supernatants are concentrated and purified by column chromatography and gel electrophoresis and labeled with radioisotopes in order to follow minute quantities. Fractions are tested in vitro and then in vivo for antitumor activity.

Progress: Mouse models were developed for lymphokine assay of antitumor activity. Significant prolongation and even cures were noted in treated mice bearing the L1210 lymphoma and the B-16 melanoma. New in vitro quantitative assays were developed for lymphotoxin and MIF, and purification of lymphotoxin biosynthetically labeled with ^{3}H-leucine, proceeded to 1000-fold greater than starting levels. Acute preclinical toxicity studies were completed and an IND has been filed with the FDA.

Significance to Cancer Research: Lymphokine preparations appear to mobilize inflammatory cells and induce tumor regression. Such preparations may be useful as adjuncts to chemotherapy, and in some cases may induce complete regression by augmenting the natural immune response of lymphocytes and macrophages to the tumor where they have previously become refractory.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Antibody-Drug Conjugates in Immunotherapy

Principal Investigator: Prof. Ruth Arnon
Name/Address Weizmann Institute
Performing Organization: Rehovot, Israel

Contract Number: NO1-CB-64049
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To explore the feasibility of an immunochemotherapeutic approach -- the use of antitumor antibodies as specific carriers of anti-cancer drugs for local delivery of the drugs to the tumor site.

Approach: Anti-cancer drugs such as daunomycin and adriamycin are attached covalently either to the IgG fraction of antitumor sera or to purified anti-tumor antibodies. The cytotoxic effect of the resulting conjugates on the tumor cells is tested both in vitro and in vivo, and compared to the effect of free drug, free antibodies, their mixtures, or conjugates of the drugs with normal immunoglobulins.

Progress: It was shown that covalent conjugation of the anti-cancer drug daunomycin to antibodies leads to a conjugate that retains to a high extent the activities of both the drug and the antibody. In vitro studies, using several murine tumor systems, have shown that the drug-antibody conjugates showed selective cytotoxic effects toward the cells recognized by the antibodies.

In the present contract year conjugates have been tested in vivo for their effect in preventing the tumor development in mice transplanted with the tumor cells. Limited beneficial effect was achieved with conjugates containing whole IgG fraction. However, conjugates of daunomycin with purified antibodies were effective, particularly when used in high doses where several experiments, though not all, resulted in survival of most or all the treated mice.

The capability of anti-Yac antibodies to "home" in vivo onto tumor cells or tissues infested by them was investigated by using double labeling technique. The results demonstrated relative accumulation of the antibodies in various tissues of tumoric mice higher than that observed in healthy controls.

Studies concerning the mechanism of action of the daunomycin conjugates consisted of in vitro experiments, demonstrating that conjugates of daunomycin and anti-Yac antibodies (containing labeled drug) are taken up readily by the relevant Yac cells, and enter into the nuclei. Lower uptake was observed when the drug was bound to normal immunoglobulin or when normal cells were used.

Significance to Cancer Research: The suggested approach is a combination of a chemotherapy and immunotherapy, in an attempt to localize the cytotoxic drug in the tumor site while maintaining its systemic concentration low. It is thus relevant to research on treatment of cancer.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Mouse Tumors Using Immunoresponsive Cells Sensitized In Vitro

Principal Investigator: Dr. Jon C. Palmer
Name/Address Wistar Institute
Performing Organization: Philadelphia, PA

Contract Number: NO1-CB-64005
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: To use mouse tumor models to evaluate the effectiveness of sensitizing host immunocompetent cells with syngeneic tumor cells by in vitro culture and the use of such sensitized cells in cancer therapy and prophylaxis.

Approach: The P815Y and L-5178Y tumors of DBA/2 mice and the A-10 tumor of A strain mice were chosen as representatives of tumors with high, intermediate, and low immunogenicity, respectively. Host immunocompetent cells are cultured with mitomycin-treated tumor cells in Marbrook cultures and in tissue culture plates using medium containing 2-mercaptoethanol. In vitro assays for cytotoxicity and for cytostasis are performed and compared with therapeutic activities of the same populations of cells transferred into mice by various routes of inoculation.

Progress: The Marbrook system of culture was compared to the more simple method of culture in tissue culture plates with small volumes of medium containing 2-mercaptoethanol; optimum conditions for generation of allogeneic and syngeneic cell-mediated responses to tumor cells were established. Besides the well-known, short-term radioactive chromium release assay for anti-tumor cytolytic activities, a colony formation in soft agar assay was developed to measure growth inhibition activities. Investigation of the variables affecting cell transfer to tumor-bearing recipients as a test for immunotherapy of tumors were initiated.

Significance to Cancer Research: Two basic problems in the treatment of cancer are (1) insufficient or inappropriate immunologic responses and (2) inadequate tumor selectivity of conventional therapies. If the goals of this project are met, it would become possible for the therapist to supplement or redirect the patient's immunologic antitumor responses or, alternatively, to maintain or restore the patient's immunologic protection against superinfection. This latter capability would permit the use of levels of radiotherapy or chemotherapy that might otherwise be prohibited because of their destructiveness to the patient's immune defenses.

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development of Therapy with Neuraminidase-Treated Tumor Cells

Principal Investigator: Dr. Richard K. Gershon
Name/Address Yale University School of Medicine
Performing Organization: New Haven, CT

Contract Number: NO1-CB-43865
Starting Date: 11/8/74 Expiration Date: 11/7/76

Goal: To increase the immunogenicity of tumor cells.

Approach: (1) To treat tumor cells with neuraminidase in an attempt to increase their immunogenicity; (2) to examine what effect the inclusion of dead tumor cells has on the immunogenicity of tumor cell inocula, and whether the rate of turnover of membrane antigens plays an important role in eliciting an effective immune response.

Progress: Protective immunity could not be elicited with neuraminidase-treated tumor cells in syngeneic transfers using the tumor lines, EL-4, L1210, and sarcoma 1. Several treatment variables were tested, including (a) the immunizing cell dose, (b) the route of immunization, (c) the time interval between immunization and challenge with untreated tumor cells, (d) the amount of neuraminidase used to remove sialic acid residues, (e) the pH at which tumor cells were treated with neuraminidase, and (f) the challenge dose of tumor cells, and none of these significantly prolonged the survival of immunized mice.

Dead cells do not inhibit the development of protective immunity, even though the immunizing inoculum is only 60% viable following neuraminidase treatment. A subcutaneous injection of 5×10^6 sarcoma 1 cells ($H\text{-}2^{kd}$) grew poorly in BDF_1 ($H\text{-}2^{b/d}$) mice if the mice had been previously injected a month earlier in the footpad with 1×10^6 viable sarcoma 1 cells. However, if 1×10^6 heat-killed sarcoma 1 cells were included together with the 1×10^6 viable sarcoma 1 cells in the original inoculum, then significant growth of the secondary subcutaneous tumor occurred before it was finally rejected.

The role that turnover of tumor membrane antigens plays in the *in vitro* generation of cytotoxic killer cells was examined since excess shedding of membrane antigens (in soluble form) or presentation of those antigens on dead cells (in particulate form) may lead either to the generation of suppressor cells or the production of blocking antibodies. Inhibiting the protein synthesis of tumor cells significantly enhances their immunogenicity in this system. Dose response studies have shown that excess antigen inhibits the development of cytotoxic cells, and we are presently investigating the possibility that suppressor cells are being generated.

Significance to Cancer Research: Neuraminidase treatment of tumor cells is an ineffective method for making them immunogenic in the tumor systems studied. Several variables were identified which make immunogenic cells less immunogenic (e.g., the presence of dead tumor cells).

Project Officer: Harriet L.G. Gordon, M.D.
Program: Tumor Immunology Program
Technical Review Group: Committee on Cancer Immunotherapy
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Maintain an Animal Holding Facility and Provide Attendant Research Services

Principal Investigator: Dr. Harold Dinkel
Name/Address Cor Bel Laboratories, Inc.
Performing Organization: Rockville, MD

Contract Number: NO1-CB-64024
Starting Date: 11/1/75 Expiration Date: 10/31/77

Goal: Maintain and breed a colony of mice and rats, and maintain a rabbit colony as specified by the Project Officer.

Approach: To house, feed, and maintain a colony of mice, rats and rabbits according to the National Research Council Standards; to perform specific technical manipulations as directed by the Project Officer; to maintain a breeding colony of specific mouse strains as required by the Project Officer.

Progress: Performance on the contract has been highly satisfactory. The mouse, rat, and rabbit colonies have been established and are being maintained according to the National Research Council Standards. Specific mouse and rat strains have been bred and breeding colonies have been established to meet the requirements of the Project Officer. The technicians assigned to the project have demonstrated their competence in segregating offspring and in keeping accurate records of the various matings performed.

Significance to Cancer Research: A small animal colony is needed to maintain important NCI intramural research concerned with neoplasia.

Project Officer: Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $178,629

CONTRACT RESEARCH SUMMARY

Title: NCI Histocompatibility Typing Center

Principal Investigator: Dr. Frances E. Ward
Name/Address Duke University
Performing Organization: Durham, NC

Contract Number: NO1-CB-53890
Starting Date: 4/1/77 Expiration Date: 3/31/78

Goal: To study HL-A, B lymphocytes and other cell surface antigens on normal and neoplastic cells and to establish correlations between individual disease categories and these antigens.

Approach: Complement-dependent cytotoxicity studies will be performed on human peripheral blood lymphocytes and leukemic cells using antisera and cells provided by the Project Officers.

Progress: In the last 12 months, about 2,500 HLA typings were performed. These typings have been of high quality and include typings of leukemia patients and parents, patients with melanoma, osteosarcoma, lung cancer, sprue, dermatitis herpetiformis, manic depressive psychoses, and schizophrenia. In addition, sera from melanoma and leukemia patients have been analyzed for antibody to HLA antigens.

In the studies of acute lymphocytic leukemia, with over 600 prospectively studied subjects, a significant perturbation of antigen frequencies at the HLA-A locus was observed, with HLA-A2 increased in frequency and several antigens decreased. This study suggests that there is a definite link between HLA antigens and acute lymphocytic leukemia of childhood.

Over 150 lung cancer patients have been studied prospectively. The data have not been analyzed yet.

Significance to Cancer Research: Development of methodologies in the identification of isolated cell surface antigens is a necessary prerequisite to the identification of tumor-specific associated antigens. Moreover, establishing correlations of malignancies and HLA antigens might not only identify factors important in prognosis, but also lead to etiologic clues.

Project Officer: Dr. G. Nicholas Rogentine, Jr., and Dr. Dean L. Mann
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 1977 Funds: $262,405

CONTRACT RESEARCH SUMMARY

Title: Cutaneous Hypersensitivity Antigen Reactions

Principal Investigator: Dr. Ariel Hollinshead
Name/Address George Washington University
Performing Organization: Washington, DC

Contract Number: NO1-CB-64022
Starting Date: 5/1/76 Expiration Date: 4/30/78

Goal: Separation and purification of human tumor-associated antigens for studies at NCI of cell-mediated immunologic reactivity to these antigens by skin tests for delayed hypersensitivity and by leukocyte migration inhibition assays. Development of antibodies to the isolated antigens and attempts to establish radioimmunoassay.

Approach: Extraction and purification of antigens from carcinoma of the breast, carcinomas of the lung, malignant melanoma, and Ewing's sarcoma. Separated antigens are provided to Laboratory of Immunodiagnosis, NCI, for detailed immunologic studies. Separated antigens are injected into rabbits and tested for presence of specific antibodies.

Progress: Large amounts of separated soluble fractions have been prepared from the MCF7 cell line derived from breast cancer and these have been extensively studied by NCI in skin tests for delayed hypersensitivity. These results indicate that some of the separated antigens elicit delayed hypersensitivity reactions in the majority of breast cancer patients and in only a small proportion of patients with other types of cancer. Thus, skin-reactive tumor-associated antigens can be prepared from a standard source and that the antigens have similar or identical specificities to those previously prepared from fresh breast cancers. A similar approach has been used with extracts from the established cell line from carcinoma of the lung. Sephadex G200 fractions from this cell line have produced skin reactions in most of the lung cancer patients but have also given reactions in half the patients with other types of cancer. Therefore, further fractionations are being performed in an attempt to separate lung tumor-associated antigens from cross-reactive antigens. Extracts have been prepared from the established cell line 5838 from Ewing's sarcoma. Separation of antigens by DEAE cellulose chromatography appears promising and large-scale studies are now being initiated.

Significance to Cancer Research: This contract provides an opportunity to study antigens which are recognized by the cell-mediated immune response of cancer patients.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $179,704

CONTRACT RESEARCH SUMMARY

Title: Systems Analysis and Informational Service in Registries of Clinical Protocols

Principal Investigator: Molly A. Wolfe
Name/Address Informatics, Inc.
Performing Organization: Rockville, MD

Contract Number: NO1-CB-53888
Starting Date: 5/2/75 Expiration Date: 5/31/78

Goal: To provide analyzed integrated information regarding ongoing clinical immunotherapy protocols to cooperating clinicians.

Approach: Standardized data accumulation and storage methods have been developed for characterizing clinical trials on immunotherapy. Protocols submitted to the Registry are analyzed, indexed, and periodically updated; an annual Compendium of these protocols is prepared and distributed to participating clinical investigators. In addition, an address file of approximately 3,000 physicians is maintained and periodically updated. Periodic reports describing current Registry activities and related topics in immunotherapy are published and distributed to those on the general mailing list.

Progress: The fourth edition of the Compendium on Tumor Immunotherapy Protocols was published in August 1976. Prior to its publication, considerable time was spent on refining both the protocol analysis form and the format of the Compendium. Updated information on protocol status was requested and received for all protocols in the Registry, and approximately fifty new protocols were analyzed. In addition, a mini-Compendium containing protocol titles and the investigator's name and address was prepared and distributed to over 2,800 clinicians. Further contract work involved the conversion of the old address file to the new Immunotherapy Registry Information System (IRIS) and to convert the old WYLBUR data sets containing the original address file to the new IRIS format.

Significance to Cancer Research: The International Registry of Tumor Immunotherapy is a valuable source of ongoing and often unpublished information on clinical trials in immunotherapy. The annual Compendium of Tumor Immunotherapy is a mechanism for a rapid exchange of current information among clinicians engaged in immunotherapy and further may encourage and aid in the design of new protocols.

Project Officer: Mrs. Susan Ficker
Program: Managed by the Tumor Immunology Program in Immunotherapy
Technical Review Group:
FY 77 Funds: Approx. $78,000 from ICRDB, OD/NCI

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator: Dr. Jack Dean
Name/Address Litton Bionetics, Inc.
Performing Organization: Kensington, MD

Contract Number: NO1-CB-63975
Starting Date: 11/1/75 Expiration Date: 8/31/78

Goal: To study the immune responses of cancer patients to tumor-associated antigens and to correlate results of different assays with each other and with clinical status of patients.

Approach: Immunological tests to evaluate and monitor the immune competence of patients and their response to tumor-associated antigens include lymphocyte stimulation by mitogens, recall antigens, and autologous tumor antigens; rosette assays for enumeration of T and their subpopulations; leukocyte migration inhibition assays against recall antigens and tumor-associated antigens; preparation of extracts for delayed hypersensitivity skin tests by NCI staff; and studies of direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity against various human tumor cell lines.

Progress: Serial testing of patients of carcinoma of the lung, breast, malignant melanoma, and Ewing's sarcoma has been continued. In lung cancer patients two immunologic assays have correlated well with clinical status and prognosis. Patients who have developed recurrent disease after surgery have depressed percentages of high affinity rosette-forming T cells and depressed lymphocyte proliferation responses to PHA and to allogeneic leukocytes. Tests of autologous proliferative responses of lung and breast cancer patients to tumor extracts have shown that approximately 50% of the patients had reactivity and that this was directed against tumor-associated antigens. Depressed autologous reactivity in breast cancer patients has been associated with radiotherapy. In studies of leukocyte migration inhibition to extracts of tumor cells and tissue culture cell lines, indirect assays confirm that the assays were measuring production of the lymphokine, leukocyte inhibitory factor, by specifically immune lymphocytes. In cytotoxicity assays, the major emphasis is on understanding normal cytotoxic reactivity and the possible presence of other types of cytotoxic cells in the blood of cancer patients. The natural cytotoxic effector cell is a subpopulation of T cells, with low affinity receptors for sheep erythrocytes and with Fc receptors. By depletion of the natural effectors cells, it can be shown that some patients have another type of effector cell which is reactive against some tumor cell lines.

Significance to Cancer Research: These studies are providing important information regarding the usefulness of various assays of cell-mediated immunity for immunodiagnosis and for monitoring the clinical course of cancer patients.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $585,513 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Biologic Studies of Solubilized Tumor Antigens

Principal Investigator: Dr. Jack Dean
Name/Address Litton Bionetics, Inc.
Performing Organization: Kensington, MD

Contract Number: NO1-CB-63987
Starting Date: 9/29/76 Expiration Date: 9/28/77

Goal: To develop a rapid, reproducible and specific assay to monitor solubilization and purification of various TSTAs. Assays must have some relationship to tumor rejection activity.

Approach: The biologic activities of detergent-solubilized and of various chromatographic fractions of plasma membranes bearing TSTA (tumor rejection activity) are studied by several methods: lymphocyte stimulation, inhibition of macrophage migration, Winn assay, and delayed hypersensitivity reactivity (in the foot pad). At the same time tumor rejection activity of these same materials is being assayed at LCB. TSTA activities of chemically induced and of DNA and RNA virus-induced syngeneic neoplasms of the mouse are being studied.

Progress: Detergent-solubilized (NP40 and deoxycholate) and partially purified fractions of TSTA following chromatography on agarose-polyacrylamide columns have shown specific activity in the agarose droplet assay for inhibition of macrophage activity and in the foot pad assay for delayed hypersensitivity reactivity. Nanogram amounts (protein) have shown activity in those fractions known to have tumor rejection activity in the syngeneic mouse. Fractions obtained from Meth-A, a chemically induced sarcoma, and from mKSA, an SV40-induced sarcoma, were specifically active whereas no specificity could be obtained in these assays using materials prepared in the same way from leukemic cells induced by RNA viruses; these latter neoplasms are continuously releasing infectious virus and this may be a complication.

Attempts will be made to determine if the above 2 assays may be used as quantitative tools; more precise dose-dependent reactivity studies will now be pursued -- in parallel studies with tumor rejection since an increase in TSTA activity was observed with further purification steps -- through lectin affinity chromatography and PAGE -- at least for one of the antigens being studied -- the TSTA of the Meth A sarcoma.

Significance to Cancer Research: (NCP Objective 3 Approach 5)
Knowledge gained through a study of the biology and chemistry of specific tumor antigens on the cell surface of neoplastic cells, transformed by RNA and DNA viruses and by carcinogenic chemicals, will hopefully lead to an understanding of the mechanisms of cancer induction and the prevention and control of neoplasms in man.

Project Officer: Dr. Lloyd W. Law
Program: Tumor Immunology Program
Technical Review Group: Immunology Support (Ad Hoc Review)
FY 77 Funds: $225,000 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Studies of the Immune Response of Mice and Rats to Tumor Antigens

Principal Investigator: Dr. Julie Djeu
Name/Address Litton Bionetics, Inc.
Performing Organization: Kensington, MD

Contract Number: NO1-CB-74093
Starting Date: 2/28/77 Expiration Date: 2/27/78

Goal: Study of immune responses of mice and rats to tumor-associated antigens. Studies on in vivo resistance to tumors and correlation with in vitro cellular and humoral immune responses.

Approach: The characteristics of the cellular immune responses to tumor-associated antigens to tumors induced by murine leukemia viruses and by murine sarcoma virus will be studied. In addition, natural cell-mediated cytotoxicity against tumors of mice and rats will be investigated. The factors influencing the levels of this reactivity and the mechanism of cytotoxicity are being studied.

Progress: The natural cell-mediated cytotoxicity in mice has been found to be augmented very substantially by inoculation of various tumor cells, viruses, BCG, and other microorganisms into mice. Similar results have been obtained in studies of rats. Recent results suggest that the natural cell-mediated cytotoxicity may be mediated by lymphocytes bearing Fc receptors, which are armed with natural antibodies in vivo. The effects of various drugs on the levels of natural cytotoxicity and on the ability to augment this reactivity have been studied, and a number of important differences have been noted in the effects of some agents on natural cytotoxicity and on specifically immune T cells. In studies of in vitro cytotoxicity against leukemia virus-infected cells, mediated by specifically immune T cells, a lack of complete H2 histocompatibility antigen restriction has been noted. Recent in vivo experiments have correlated with the in vitro results, indicating that effective resistance against tumor growth can be produced by allogeneic as well as syngeneic tumor cells.

Significance to Cancer Research: (NCP Objective 6 Approach 4)
Understanding of the relationship of in vitro assays to host resistance to tumors is an essential element in properly using these assays to monitor responses to immunotherapy and to understand the role of various immune mechanisms in resistance to tumor growth. The recent findings with natural cell-mediated immunity require reevaluation of the types of immune mechanisms involved in immunologic surveillance.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $259,608

CONTRACT RESEARCH SUMMARY

Title: Melanoma Cell Vaccine and *In Vitro* Assays for Humoral and Cellular Cytotoxicity

Principal Investigator:	Dr. Edwin Matthews
Name/Address	Litton Bionetics, Inc.
Performing Organization:	Kensington, MD

Contract Number: NO1-CB-53916
Starting Date: May 9, 1975 Expiration Date: 5/10/78

Goal: To prepare cultured melanoma cells for immunotherapy vaccine and carry out *in vitro* cytotoxicity tests to measure response of patients to the vaccine.

Approach: (1) Grow human melanoma cells free of contaminants, treat cells with neuraminidase, freeze under sterile conditions, and provide cells as needed for human immunotherapy. (2) Carry out tests for complement-dependent antibody, T-cell activity, direct cell-mediated cytotoxicity and memory for direct cell-mediated cytotoxicity.

Progress: Between May 1975 and December 1976, 11 batches of pooled vaccine were prepared from human melanoma cell lines, UCLA SM-6, UCLA SM-12, and UCLA SM-14. All of these vaccine preparations have met quality control tests and have been used in the melanoma immunotherapy protocol. Between May 1975 and December 1976, approximately 1,880 assays for cellular immunity and 3,800 assays for humoral immunity were carried out using blood samples from patients receiving the vaccine, patients in the control groups of the protocol, and normal donors. Results have shown that 22 of 23 patients receiving the melanoma vaccine have developed humoral immunity against one or more of the vaccine cell lines within two months after treatment was started. Only one of 58 patients in the control group appeared to develop humoral immunity against one of the vaccine cell lines; this patient was in a control group receiving BCG alone and, in addition, had significant humoral immune activity prior to initiation of BCG treatment. Results of tests for cellular immunity have demonstrated that 5 of 5 patients receiving the melanoma vaccine and tested frequently within the first 4 months after initiation of therapy developed significant immune activity. Fourteen of the 18 patients receiving the melanoma vaccine who were tested less frequently during the course of immunotherapy also developed significant immune activity against one of the vaccine cell lines. Whether quantitative levels of immune responses to the immunotherapy correlate with clinical courses of the disease is not yet clear.

Significance to Cancer Research: (NCP Objective 6 Approach 1) The melanoma cell line vaccine will be used in a clinical immunotherapy trial.

Project Officer: Dr. William D. Terry and Dr. John R. Wunderlich
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $335,444

CONTRACT RESEARCH SUMMARY

Title: Rhesus Monkey Histocompatibility Studies

Principal Investigator: Dr. Bruce Maurer
Name/Address Litton Bionetics, Inc.
Performing Organization: Kensington, MD

Contract Number: NO1-CB-64016
Starting Date: 11/1/75 Expiration Date: 10/31/77

Goal: To establish and maintain a colony of pedigreed rhesus monkey families for the study of the genetics of transplantation and as an eventual source of donor-recipient pairs for transplant experiments.

Approach: Sera raised by allo-immunization of selected monkeys are analyzed for serologically defined antigens by microcytotoxicity typing of families and large random unrelated populations. Monkeys are typed for MLC locus antigens by performing mixed lymphocyte cultures using as stimulators the three available monkeys homozygous at this locus. The method of Mann et al. is used to identify B lymphocyte alloantigens serologically. Breeding is designed to enlarge existing families of full siblings.

Progress: Analysis of about 150 alloantisera has resulted in improved definition or new identification of a total of 20 RhL-A alloantigens. During the current contract year, analysis of several new sera by methods of population genetics and by absorption led to the identification of three of these 20. LD typing using MLC locus homozygotes has resulted in identification of two antigens. Optimization of a system for primed lymphocyte typing (PLT) has been initiated. This method potentially permits identification within a family of all LD antigens responsible for stimulation and proliferation of lymphocytes mixed in culture. The homozygous typing cell method requires the birth of an RhL-A homozygote for each antigen to be identified. Typing of a random population of monkeys with a panel of 57 sera detecting antigens exclusively present on B lymphocytes has been completed. Typing of sera of families for properdin factor B (Bf) in collaboration with Dr. Chester Alper has been started in order to resolve some ambiguities in the RhL-A haplotyping of several families and to identify several possible RhL-A recombinants. Breeding has resulted in 7 live births and 5 currently undelivered pregnancies.

Significance to Cancer Research: (NCP Objective 6.1A Approach 6.6)
Bone marrow transplantation may allow more intensive cancer chemo-, radio- or immunotherapy than is presently possible. Transplantation of other organs may be useful for replacement of tumor-ravaged organs.

Project Officer: Dr. J. R. Neefe, Jr..
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $223,304

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation, and Preservation of Plasma Cell Tumors in Mice

Principal Investigator: Dr. Kyle Sibinovic
Name/Address
Performing Organization: Litton Bionetics, Inc.
Bethesda, MD

Contract Number: NO1-CB-92141
Starting Date: 6/23/69 Expiration Date: 1/31/78

Goal: Transplantation, preservation, induction, and shipping of plasmacytomas, T- and B-cell lymphomas in mice. Breeding of rare strains of mice, hybrids, and wild mice.

Approach: Contractor maintains in transplant, freezes, and preserves about 500 tumor lines of plasmacytomas, B- and T-cell lymphocytic tumors. (Some other tumors are also maintained). These tumors, many of which are standard reference tumors, are shipped to other investigators. Plasmacytomas are used as a specific source of (1) homogenous immunoglobulins representing the major heavy and light chain classes in the mouse; (2) genetically polymorphic forms of homogeneous immunoglobulin; and (3) antigen-binding myeloma proteins. Contractor transplants these tumors and supplies ascites, immunoabsorbed purified protein to Project Officer and other investigators. Colonies of wild mice and Mus species, Caroli, Cervicolor, Dunni and Shortridgei are maintained. These mice are used by Project Officer and limited numbers are supplied to others. The contractor breeds and supplies hybrid mice for genetic studies.

Progress: The contractor has maintained 1,150 tumor lines including 242 new tumors; and has handled 1,809 shipments for 340 outside investigators, 804 tumor and ascites shipments for the Project Officer. Purified myeloma proteins and monospecific antisera were prepared and used in a variety of test systems. The contractor maintains a large mouse colony with a breeding nucleus of 62 recombinant inbred strains and congenic lines and approximately 12 to 15 hybrids. Two new crossover stocks were found (11235 and 11364) and are being progeny tested. In addition, the contractor maintains approximately 31 mouse strains, prepares inbred, cross-bred and backcross mice and ships products to outside investigators. Antisera for histocompatibility typing are being prepared and testing for various markers is under way. The computer program was upgraded and the master Tumor file, Quality Control file, Ascites file, and Investigator Report were refined.

Significance for Cancer Research: Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Dr. Michael Potter and Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $425,331

CONTRACT RESEARCH SUMMARY

Title: National Cancer Institute Immunodiagnostic Reference Center

Principal Investigator: Dr. Kenneth Kortright
Name/Address Meloy Laboratories, Inc.
Performing Organization: Springfield, VA

Contract Number: NO1-CB-63976
Starting Date: 7/11/75 Expiration Date: 7/10/78

Goal: To develop new assays for tumor-associated antigens, to use immunochemical techniques for diagnosis of cancer, and to evaluate humoral immune responsiveness of patients with cancer.

Approach: Sensitive radioimmunoassays are being used to quantitate human, monkey, and mouse alpha-fetoprotein and human chorionic gonadotropin in serum. These assays are being evaluated as diagnostic techniques for testicular, ovarian, and hepatocellular cancer and to define the response to therapy. In addition, alpha-fetoprotein is being purified to define its immunosuppressive properties. Human lung tumors are being fractionated to isolate an antigen reactive with antisera against human lung tumors. In addition, the immunodiagnostic center performs tests for humoral competence of patients with cancer or immunodeficiency diseases. Finally, in cooperation with WHO the immunodiagnostic center serves as a distribution outlet for provision of reference material for human serum immunoglobulin quantitation.

Progress: In a prospective study of patients with testicular tumors, AFP and HCG assays have been of great value as an adjunct to the diagnosis of a testicular mass, in staging germ cell tumors of the testis as a prognostic tool, and as a tool to detect residual tumor cells in a patient receiving therapy for his tumor. In over 30 patients being treated for testicular cancer but with no clinical evidence of residual disease, a positive marker always reflected occult residual tumor. There is, however, an approximately 11% false negative rate using these markers. Thirty mg of alpha-fetoprotein has been purified from human cord plasma and is being used in studies of the immunosuppressive properties of alpha-fetoprotein. The human lung tumor extracts have been isolated that react with an antiserum against human lung tumors but not against normal human lung tissue. These represent preliminary observations directed toward establishing an immunodiagnostic test for human lung cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A center with the capability of performing multiple immunodiagnostic tests will help to rapidly establish the usefulness of these tests in the diagnosis of cancer or as methods for monitoring patients during and after therapy.

Project Officer: Dr. Thomas A. Waldmann, Dr. R. Michael Blaese, Dr. Robert McIntire
Program: Tumor Immunology Program
Technical Review Group:
FY 77 Funds: $238,538 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Preparation of Reagent Antisera and Antigens

Principal Investigator: Dr. Howard M. Grey
Name/Address National Jewish Hospital and Research
Performing Organization: Center
Denver, CO

Contract Number: NO1-CB-53912
Starting Date: 5/16/75 Expiration Date: 5/15/78

Goal: Preparation of monospecific fluorescent-isomer conjugated IgG $F(ab')_2$ antibodies to all heavy and light chain classes of human and mouse immunoglobulins and human T lymphocytes.

Approach: All classes of human and mouse heavy and light chains will be purified by immunochemical techniques. These will be used to immunize animals and will be conjugated to Sepharose beads. The resulting antisera will be absorbed with appropriate conjugated beads to render them monospecific. The IgG fractions of such antisera will be isolated, enzyme digested to make $F(ab')_2$ fragments, and conjugated to fluorescent isomers. A variety of cell types will be used for both immunization and absorption to make human T-cell-specific antisera. The antibodies will then be further processed as for the anti-Ig reagents.

Progress: Antibodies as described above have been received to all human immunoglobulin classes except epsilon. This as well as additional amounts of μ, α, and δ should be completed shortly. Rabbits have been immunized with monkey thymocytes and the resultant sera are being tested for antibody activity on human lymphocytes.

The antibodies to the mouse Ig classes have been raised and are in various stages of processing (absorption, digestion, or conjugation). Completion of these reagents within 6 months is expected. Hyperimmune antisera to rhesus thymocytes have been raised and absorption and testing of specificity will begin shortly. Immunization of rabbits with human brain is also to scheduled to begin.

Significance to Cancer Research: To provide specialized antisera reuqired for cell surface studies important in the Baseline Information Flow of the Tumor Immunology Program.

Project Officer: Dr. Howard B. Dickler and Dr. Warren Strober
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 77 Funds: $70,831

ANNUAL REPORT
Breast Cancer Program Coordinating Branch
July 1, 1976 - September 30, 1977

The basic responsibility of the Branch continues to be one of providing support for the overall functioning of the program of the Breast Cancer Task Force (BCTF) and to organize and to monitor the contract projects funded by the Task Force. Changes made in the organization and the operations of the Task Force and its review committees the previous year were carried out in this fiscal year. The four technical review committees met bimonthly through May 1977 when travel funds were insufficient to complete the scheduled meetings for July and September. Plans are presently underway to incorporate the four review committees into one chartered group to be known as the Breast Cancer Task Force. There will be sufficient scientific expertise within the committee in order to form working groups that will continue to provide program recommendations in the areas of diagnosis, epidemiology, experimental biology and treatment.

At the beginning of the Fiscal Year 1978 the Task Force will have over 85 contracts and six Cancer Research Emphasis Grants funded. In FY-1977 the four review groups advertised 27 new projects, approximately 250 proposals were received in response, and reviewed by the members of the four committees. About 12 unsolicited proposals and "letters of intent" were likewise received and evaluated, only two were approved and neither has been funded. One was to have been supported in this year, however, the principal investigator moved to another university. Thirty-two new projects have been initiated in FY-1977. The program continues to phase out 20 to 30 percent of the contracts yearly and to replace them with new projects.

The goals of the BCTF, namely, determining the etiology of the disease, identifying high risk populations, improving diagnostic methods, finding new treatment modalities, and preventing the occurrence and recurrence of the disease, are related to the subject areas to be enumerated below. An individual contract may, and often does, contribute information to more than one subject area. Contracts related to the various areas are listed by their respective numbers.

I. Genetic, environmental, dietetic and other factors in breast cancer: Related contract projects are: a. Genetic markers as tools to identify high and low risk cancer families - 43940, 44003, 44004. b. Nutritional and genetic factors - 43973, 53883. c. Morphological aspects - 53854, 53968. d. Breast cancer risk in relation to estrogens - 44002, 53884, 74091. e. Structure and function of epithelial basal lamina - 53903.

II. Predicting breast cancer behavior. Related contracts are: a. Epidemiology of benign breast disease - 74102. b. Epidemiology of minimal breast cancer in initially asymptomatic female - 74103. c. Epidemiological characteristics of medullary and lobular breast cancer - 63997. d. Spectrum of risk factors for pre- and post-menopausal breast disease - 63996, 63998. c. Prognostic factors for disseminated cancer in patients with early invasive disease - 74097. f. Occult breast cancer metastasis in axillary lymph nodes - 74110. g. Endocrine therapy plus chemotherapy in patients - 64000.

III. Isolation, cultivation and characterization of normal and neoplastic mammary tissue. Related contract projects are: a. Conditions for growth and passage of primary cultures of normal mammary epithelium - 74094. b. Isolate and cultivate mammary cells from milk - 33898. c. Isolate and cultivate mammary carcinoma cells from pleural effusion - 23869. d. Dissociation of mammary epithelial cells from solid tissue - 33861. d. Characterize mammary cells by isoenzyme pattern - 33880. e. Transplant human mammary carcinomas in athymic nude mice - 33856. f. Assess human preneoplastic lesions in athymic nudes - 43908. g. Nature of serum factors necessary for growth of mammary epithelium - 23875.

IV. Dependence on, and response to hormones. Related contract projects are: a. Role of androgens in morphogenesis of mammary gland and hypothalamic-pituitary axis - 33883, 33907. b. Detection, isolation and quantitation of hormone binding proteins - 23862, 23859, 43969, 43905, 43990, 53905, 23873, 64000. c. Steroid sulfurylation - 43867. d. Effects of hormones on assembly and function of mammary chromatin - 43866. e. Relationship between prolactin and mammary tissue - 63985, 23863, 23859. f. Clinical trials with hormone therapy - 43991, 53851, 64000. g. Estriol dynamics - 33902. h. Relation of thyroid function to growth and development of mammary gland - 74107.

V. Detection and diagnosis of early breast cancer: Related contracts are: a. X-ray diagnosis—comparison of xero—to film mammography clinical and physical aspects - 43993, 44011, 44012; computerized tomography - 64047. b. Thermography and mass screening - 43869. c. Ultrasound, quantitative imagining - 64041. d. Pathology (high risk groups) - 43871. e. Biochemical studies for diagnostic parameters - 33882, 53853. f. Immunological cross-reactions between breast cancer and known or refuted mammary tumor viruses - 74087. g. Markers of breast cancer presence: 1) Longitudinal studies of biological markers - 74086. 2) Serum sample bank - 74137, 74138. h. Risk associated with diagnosis of non-invasive diseases and preneoplasia - 74098.

VI. Intrinsic and extrinsic factors affecting the defenses of the host and growth potential of mammary neoplasia. Related contracts are: a. Role of fat pad mammary pathophysiology: 1) interaction of stromal cells and mammary epithelium - 43907; 2) glucosaminoglycans on basal membrane - 53903; 3) mesenchymal cell types interacting with mammary epithelium - 63986. b. Transfer of chromosomal DNA into neoplastic cells or from neoplastic to susceptible cells - 43904, 53904. c. Studies of cell kinetics and prediction of growth potential - 33861, 33903, 43899, 74083. d. 1) Evaluation of therapeutic regimen in animal tumor model systems - 43914; 2) Prevention of the formation and progression of preneoplasia - 74090. e. Effects on rodent breast carcinomas of phenylalanine ammonia lyase - 43906. f. Age and endocrine environment as interrelated factors, changing sensitivity to carcinogens of mammary gland as a function of the physiology of the host - 74105. g. Characterization and possible quantitation of tissue changes produced by completed pregnancy in testing mammary gland - 74101. h. Effect of antigenic material produced by tumor on the host, inhibition of cellular immunity - 33905. i. Movement of materials across the endothelium, tumor microcirculation - 63981. j. Relation of intestinal flora to the human disease - 74104.

VII. Metastasis. Related contracts are: a. Capacity of mammary tumors to metastasize - 63980. b. Development of macromolecular ligands able to bind and separate plasma membrane components - 33908. c. Properties of normal and neoplastic mammary cell plasma membranes - 63983, 63984, 64069, four Cancer Research Emphasis Grants concern studies of mammary cell membranes. d. Osteotropism in mammary carcinoma metastasis - 63980. e. Destruction or damage of endothelium by metastatic cells or stimulation to proliferate in angiogenic activity - 74089. f. Influence of mammary neoplastic cells on the formation and absorption of collagen - 74109. g. Steroid sulfotransferase and estrogen binding levels as markers of disseminated metastases - 43900. h. Post-surgical treatment of human breast cancer: 1) patients with metastases in axillary nodes (Stage II) - 43917, 23876, 33899, 43990, 53917; 2) patients with distant metastases - 43991, 53851, 64000. i. Frequency of occult metastases and correlation with treatment failure - 74110. j. LDH isoenzyme patterns in advanced disease - 53855. k. Immunological aspects of advanced disease - 23871.

VIII. Support to total program. Contracts in this area are: a. Mammary cells and tissue bank; solid tumors, human cell populations, human and rat α-lactalbumin and their respective antisera - 74175. b. Estrogen binding protein assay - 23862, 43867. c. Breast cancer tissue supply - 23867. d. Data collection and evaluation for the program of the Treatment Committee - 74140.

ACTIVITIES

November 22-23, 1976: Breast Cancer: A Report to the Profession, 1976

July 7, September 8, 1976 and January 12, 1977: BCTF Workshops (one day each)

PUBLICATIONS

Gullino, P. M., Taylor, D. J., and Levine, B. S.: Breast Cancer Task Force Program and Related Projects. Bethesda, MD. January 1977.

Carbone, P. P. and Sears, Mary E.: Breast Cancer: Suggested Protocol Guidelines for Combination Chemotherapy Trials and for Combined Modality Trials. DHEW Publication No. (NIH) 77-1192.

CONTRACT RESEARCH SUMMARY

Title: Ultrasound Mammography

Principal Investigator: Dr. Gilbert Baum
Name/Address
Performing Organization: Albert Einstein College of Medicine
Bronx, NY

Contract Number: NO1-CB-23872
Starting Date: 6/20/72 Expiration Date: 12/31/76

Goal: To develop the criteria for detection and for the differential diagnosis of breast cancer employing ultrasound, a noninvasive, painless and harmless modality.

Approach: Patients who are to undergo breast surgery are referred into the study on a random basis from breast clinics and surgeons. Scanning is performed on each breast, where the unaffected breast is used as the control. Scanning is begun at the top of the breast and continues down in vertical 3 mm steps. The ultrasonograms are recorded on 35 mm half frame film (black and white) and the films from both breasts are simultaneously projected for comparison. Additional film analyses are conducted using an isophotodensitometer, which converts the grey scale of the films into specific colors, in which each color indicates a specific range of amplitude. Finally, the ultrasound, clinical and x-ray diagnoses are compared against the pathological report, and statistical evaluations are conducted.

Progress: A new ultrasonoscope has been completed and is being tried on patients to establish an examination base level for studying patient unknowns. The new scanner possesses additional scanner motions and a totally new electronic signal system. Initial patient scans show that the new system overcomes the artifacts produced by sector scanning. The ultrasound mammograms of normal and diseased breasts produced by the new unit are being compared with the mammograms produced by the earlier generation ultrasonoscope to establish a new set of diagnostic criteria.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/21/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunological Studies of Human Mammary Carcinoma

Principal Investigator: Dr. Donald M. Marcus
Name/Address Albert Einstein College of Medicine
Performing Organization: Bronx, NY

Contract Number: NO1-CB-23871
Starting Date: 6/20/72 Expiration Date: 6/18/78

Goal: (1) To identify antigens associated with breast cancer and to develop a quantitative radioimmunoassay (RIA) for use in immunodiagnosis of breast cancer; and (2) to study the correlation between in vitro assays for cell-mediated immunity to breast tumors and the clinical status and course of patients, and to identify antigens that elicit cellular immunity.

Approach: (1) To develop antisera specific for tumor-associated antigens, purify the antigens by immunoadsorbent and biochemical techniques and to use the purified antigens in a double-antibody RIA. (2) To make extracts of primary breast tumors obtained at the time of surgical resection and to test autologous and allogeneic extracts against the lymphocytes of the patient by three assays: (a) a viral plaque assay developed by Dr. B. Bloom; (b) inhibition of leukocyte migration; and (c) incorporation of ^{3}H-thymidine.

Progress: (1) Serum ferritin values are elevated in many patients with breast cancer. The contractor is collaborating with the NCI in evaluating ferritin and other markers in the immunodiagnosis of breast cancer, and recently assayed approximately 750 coded sera. The statistical analysis is not complete, but the combination of ferritin and CEA appears to be very promising. Acidic isoferritins have antigenic determinants that differ from normal adult liver and spleen ferritins. An RIA for acidic isoferritins was devised to determine if this assay detects cases of breast cancer that are not detected by our standard ferritin RIA.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: K. Robert McIntire, M.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/5/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $110,738

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography -- A Comparative Study

Principal Investigator: Dr. Feargus O'Foghludha
Name/Address Duke University
Performing Organization: Durham, NC

Contract Number: NO1-CB-44011
Starting Date: 6/28/74 Expiration Date: 6/26/78

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: Duke University's portion of this inter-institutional study will be subdivided into physics and clinical areas. Duke physics staff will provide spectrometric services so that xerographic and film modalities can be compared under known spectral conditions. The effects of the radiation spectrum, as modified by kV, filtration and waveform, on image quality and on dose will also be studied, and the results will be used to determine optimum techniques. In the clinical area, staff will participate in subjective inter-institutional evaluation. In addition, cephalocaudal images made at Duke will be furnished to the Diagnostic Radiology Committee of the National Cancer Institute for review by an advisory board.

Progress: The spectroscopic equipment has been computerized and programmed to extract exposure/keV, total exposure, and HVT from raw pulse-height spectra. By this means exposure and HVT have been measured for both W and Mo anodes as a function of kV, filter thickness and voltage ripple. Depth-dose figures in lucite for various incident spectra have also been determined. As an aid in determining integral dose, a stereophotogrammetric method of breast volume determination has been devised and a rapid-scanning reader for large-area TLD dosimeters has been developed. Experiments on rhodium-anode tubes have been carried out, demonstrating the output and patient-dosage superiority of such tubes in comparison with Mo anodes. Clinical studies on 274 patients have been performed under subcontract at Hermann Hospital and are continuing.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/31/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography -- A Comparative Study

Principal Investigator: Dr. Philip Strax
Name/Address Guttman Institute
Performing Organization: New York, NY

Contract Number: NO1-CB-44012
Starting Date: 6/28/74 Expiration Date: 6/27/78

Goal: To evaluate the relative merit of conventional radiography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: Characteristics of each process are being assessed by way of radiographic contrast, latitude, sensitivity and modulation transfer function.

Progress: A total of 1,330 women have been entered into the study since its inception. Since November 1, 1976, Kodak AA type film has been eliminated because of the comparatively high radiation dose required. All films are in process of evaluation as per image evaluation form created for this purpose. Radiation outputs at M.D. Anderson Hospital have been obtained (air measurements: R/100 MAs). A Victoreen 415 A intercomparison chamber was used with an electrometer and digital read out. 415A was recalibrated at Anderson in terms of R/Coul. Measurements were taken for tungsten tube (no added filtration), and a moly tube (0.03 mm Mo added) for the AA, low dose and xeroradiographic techniques. Thermoluminescent dosimetry in the low energy range for 0.1 to 25 R has been conducted and skin entrance doses with both tungsten and molybdenum targets reconfirmed.

The latest physics effort has been directed to obtaining depth dose data employing several 3M phantoms. One of these phantoms has been altered so as to receive thermoluminescent dosimeters at various depths. A second 3M phantom has had a number of slices made along the short axis so as to interpose the various receptors for depth dose values for an average breast. These latter exposures are "read" on an automatic film densitometer.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/31/77
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $17,000

CONTRACT RESEARCH SUMMARY

Title: Immunologic Cross Reactions Between Breast Cancer and Mammary Tumor Virus

Principal Investigator: Grace B. Cannon, Ph.D.
Name/Address Litton Bionetics, Inc.
Performing Organization: Kensington, Maryland 20795

Contract Number: NO1-CB-74087
Starting Date: 7/1/77 Expiration Date: 6/30/78

Goal: To perform the leukocyte migration inhibition (LMI) assay for the detection of mouse mammary tumor virus (MMTV) and associated (gp-52) antigens to ascertain the usefulness of CMI assays for the early diagnosis, monitoring and prognostic predictability of breast cancer.

Approach: Breast cancer patients (prior to biopsy and post mastectomy), benign disease patients (pre and post biopsy), patients with other types of cancers, patients with gynecological disease and healthy female controls will be tested under code with MMTV and gp-52. Antigen controls will be gp-70, other coded glycoproteins, glycoprotein sub-units, a 3M KCl extract of MCF-7 and an extract of a lymphocyte pool from several normal individuals, and mumps antigen.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 1/11/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $118,337

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Computerized Tomographic Mammography

Principal Investigator: Dr. John J. Gisvold
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-64047
Starting Date: 6/30/76 Expiration Date: 12/29/77

Goal: To clinically evaluate the ability of computerized tomographic mammography (CTM) to detect early breast abnormalities and to compare its effectiveness with physical examination and mammography in individual differential diagnosis.

Approach: A prototype computerized tomographic machine specifically designed to examine the breast has been built by GE and is now in a functional state at Mayo. As many patients as possible who are scheduled for breast biopsy and who have had mammography and CT mammography will comprise the study population which will determine if CT mammography is a feasible diagnostic examination technique for detection of early breast cancer and at a lower radiation dosage than is used for current mammographic techniques. For the remainder of the study patients will be examined both before and after the intravenous injection of iodinated contrast material.

Progress: A previous summary listed our experience with CTM done without the injection of iodinated contrast material. Now CTM evaluation is done on all patients both before and after the injection of contrast material. Results are available for a total of 123 operated lesions, 68 benign and 55 malignant. Of the malignancies, mammography correctly identified 87.3% of these lesions and CTM correctly identified 85.5% of the lesions with contrast injection. Only one out of the 55 malignant lesions was missed by both modalities.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/10/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Breast Diagnosis: Quantitative Imaging by Ultrasound

Principal Investigator: Dr. James F. Greenleaf
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-64041
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To determine if images representing quantitative distribution of basic mechanical tissue properties or acoustic parameters of tissue such as velocity, attenuation, reflection, impedance and scattering can be useful in breast cancer diagnosis and of more value than images representing tissue interfaces and geometries such as B-scans.

Approach: A transverse radial scanning system has been designed and constructed which acquires ultrasound data from excised tissues and patients in several modes including echos for compound B-scans, time-of-flight and attenuation data for mathematical reconstruction. The ability of acoustic tissue characterization to detect and distinguish excised normal and diseased tissues is being studied by comparing results from visual evaluation and computer-aided feature extraction and pattern recognition of the various acoustic images with computer encoded histologic characteristics obtained from microscopic evaluation of tissue sections removed from the intact organ. Initial studies have begun of normal and diseased breasts in vivo in selected patients and the resulting acoustic images are being compared to histology obtained from breasts of those women who undergo mastectomy.

Progress: Signal preprocessing systems and scanner control electronics have been designed and constructed. Software programs for data acquisition analysis and display are being written and documented. Temperature dependence of acoustic speed in various tissue components of breast tissue is being studied. Protocols for acquisition of patient data and subsequent tissue analysis are being developed. The first patients have been scanned.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D., R. Quentin Blackwell, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $137,000

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator: Drs. George Blumenschein and Relda Cailleau
Name/Address M.D. Anderson Hospital and Tumor Institute
Performing Organization: Houston, TX

Contract Number: NO1-CB-23869
Starting Date: 6/1/72 Expiration Date: 12/31/76

Goal: To establish tumor cell lines from solid tumors or pleural effusions from breast cancer patients; to supply these cells, effusions and/or blood samples to collaborators for immunodiagnostic assays or other studies; to characterize the lines we have established by chromosomal, biochemical or immunological methods.

Approach: Cells from fresh pleural effusions are concentrated by centrifugation, hemolyzed, neutralized, and resuspended in various media. Aliquots are initially taken for chromosome analysis, staining and electron microscopic study. Primary cultures and established cell lines are frozen in glycerin or DMSO at -70° or in liquid nitrogen for storage or later distribution.

Progress: While no cell lines have been grown successfully from solid primary breast carcinoma, 15 lines have been established from metastases. Thirteen lines are from pleural effusions, one from a pericardial fluid and one from a brain metastasis. An abnormal extra-long chromosome present in a majority of our lines was studied by the G-banding chromosome technique, and the presence of a 1q marker was noted. Further studies on a total of 9 lines showed the presence of this 1q marker in every line. The 1q marker may be attached to any one of several chromosomes (3q, 5q, 7q, 11q, 12q) (Ref. in Cytogenet. Cell Genet. 17: 231-35, 1976). Inoculation of cells into nude mice has yielded 5 positive tumors out of 9 lines tested. Their morphological characteristics resemble those of the original tumors. All tests for G6PD (with one exception) show the lines to be Type B. The exception presents other unusual isozyme patterns and does not resemble HeLa cells. Immunological studies using the most prolific lines (MDA-MB 157, 231) show antigenic properties in both humoral and cell-mediated assays. Tests for estrogen binding on 6 lines showed only one line giving positive results (MDA-MB-134). Nine cell lines have been given to Dr. Jensen of the Breast Tumor Cell Bank, and 13 lines have been sent to Dr. Jorgen Fogh of Sloan-Kettering. Many of the lines have been sent to various laboratories throughout the United States, Canada, and Europe.

Significance for Cancer Research (NCP Objective 5 Approach 5, 6)

Project Officer: Ronald B. Herberman, M.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 7/22/75
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography -- A Comparative Study

Principal Investigator: Dr. Gerald D. Dodd
Name/Address
Performing Organization: M.D. Anderson Hospital and Tumor Institute
Houston, TX

Contract Number: NO1-CB-43993
Starting Date: 6/28/74 Expiration Date: 6/27/78

Goal: To evaluate the relative merit of conventional film mammography vs. xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: This project is being carried out among four institutions: The University of Texas System Cancer Center, M.D. Anderson Hospital; Stella and Charles Guttman Breast Diagnostic Institute; Duke University; and Hermann Hospital. Mammographic image formation and information-transmitting characteristics of each process will be assessed by way of radiographic contrast, latitude, sensitivity and modulation transfer function. An advisory and monitoring body set up by the Diagnostic Radiology Committee of NCI will review the technical quality of the work performed by the participating institutions. Five hundred patients per year over 40 years of age will be studied by each institution.

Progress: In the third year of the contract period at M.D. Anderson, the clinical protocol of examining all women included in the study on an overhead tungsten anode mammographic tube with three image receptors--Kodak AA non-screen film, DuPont LoDose screen film, and xeroradiographic plates--was continued until January 1977, at which time 650 women had been entered under the new protocol (established July 1975). No additional patients were entered until after it was determined to delete the Kodak AA examination from any further studies. Since April 1977, there have been 75 women entered into the contract study. Preliminary statistical resuits indicate little difference in the diagnostic accuracy among the three modalities. Statistically significant differences have been observed in the objective physical parameters. Modulation transfer functions and Wiener spectral measurements of the Dupont LoDose system and Kodak AA direct X-ray film were obtained for resolution and noise comparisons. At the present time methods have not been established to determine the MTF and Wiener spectrum of the xeroradiographic system in order to make a direct comparison with the LoDose system and AA film. However, we have used a specially designed test object to compare resolution difference between LoDose, AA film and the xeroradiographic system.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/31/77
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Tissue Supply

Principal Investigator: Dr. Paul O'Brien
Name/Address Medical University of South Carolina
Performing Organization: Charleston, SC

Contract Number: NO1-CB-23867
Starting Date: 6/30/72 Expiration Date: 6/29/78

Goal: To obtain tissue and blood specimens from patients with breast cancer for use in immunodiagnostic assays.

Approach: At the time of surgery, obtain tumor and normal breast tissue. Obtain serum and peripheral blood lymphocytes before surgical therapy and at regular intervals thereafter. These specimens are shipped promptly to other investigators in the program for use in immunodiagnostic assays.

Progress: During the period from August 1, 1976 to April 30, 1977, there were approximately 34 preoperative blood specimens from patients with malignant breast tumors, 34 malignant breast tumors, 85 blood samples from patients with benign breast diseases, 54 follow-up blood specimens on patients with malignant tumors, and 149 blood samples sent for special studies on patients with breast cancer. There were 5 benign breast tumor samples sent during this period of time. Benign blood samples and tumors were shipped only upon request.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ronald B. Herberman, M.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $55,000

CONTRACT RESEARCH SUMMARY

Title: Prognostic Factors for Disseminated Cancer in Patients with Early Breast Cancer

Principal Investigator: Paul Peter Rosen, M.D.
Name/Address Memorial Hospital
Performing Organization: New York, New York 10021

Contract Number: NO1-CB-74097
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To determine the frequency of subsequent metastatic breast cancer in a group of females with "early" invasive carcinoma and to identify those clinical and/or pathologic features or groups of features that are indicators of a high or low risk for dissemination of carcinoma.

Approach: A study population of 250 women with primary infiltrating breast carcinomas 1.0-2.0 cm in diameter, also a subset of 100 women with malignant tumors 1 cm or less in diameter will be identified in a retrospective study (1960-1968) and followed for at least 10 years. These patients will have been treated by at least a modified radical mastectomy and been found to have negative lymph nodes. The relationships observed between pathologic and clinical data and survival will be evaluated in order to identify those factors associated with a high and low frequency of recurrence.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/12/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $70,031

CONTRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator: Morton K. Schwartz, Ph.D.
Name/Address Memorial Sloan-Kettering
Performing Organization: New York, New York 10021

Contract Number: NO1-CB-74206
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To assay human breast cancer tissue and sera prior to mastectomy and throughout the clinical course of the disease for potential markers to determine how effective such markers would be for early detection of breast cancer and its recurrence and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Serum and tissue specimens from patients entering for diagnosis and for primary therapy will be assayed for carcinoembryonic antigen (CEA), sialytransferase, phosphohexose isomerase (PHI) and spermine, ferritin, lactic dehydrogenase (LDH) and its isoenzymes, glucose-6-phosphate dehydrogenase (G6PD)-tissue only as a reference, β-hCG, γ-glutamyltranspeptidase and α-fetoprotein and IgA. Total protein and DNA will also be determined to serve as points of reference. Sera specimens will be collected prior to surgery, before discharge and then sequentially, every three months the first year and every six months thereafter. For those with no recurrences, specimens will be collected on a yearly basis. Tissue will be obtained at time of biopsy and mastectomy. Should recurrences follow, metastastic tissue would undergo the same testing procedure. During the first year approximately 150-180 patients with primary breast cancer and an additional 50 patients with benign disease will be entered into the study. Twenty to twenty-five specimens of normal breast tissue (from autopsy specimens) will also be assayed. Tissue and serum biochemical-immunochemical data will be compared and changes and differences related to the clinical status of the patient.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/12/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $97,300

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Serum LDH Isoenzymes in Women with Breast Tumors

Principal Investigator: Dr. Ervin J. Hawrylewicz
Name/Address Mercy Hospital and Medical Center
Performing Organization: Chicago, IL

Contract Number: NO1-CB-53855
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To determine if a highly reproducible LDH isoenzyme test can be utilized as a diagnostic marker for breast cancer.

Approach: Blood will be drawn on 800 patients with breast disease, including malignant and benign tumors, and patients with other gynecological problems. Emphasis will be placed on securing serum samples prior to surgery. Patient cooperation will be requested when presenting for mammographic examination, and when available, LDH isoenzyme patterns will be correlated with the mammogram report. Longitudinal sampling following the course of disease will be pursued. In addition to these patients, blood from 200 women clinically free of breast disease will be obtained as control. All samples will be coded immediately; laboratory personnel will not have access to the code. Serum LDH isoenzyme patterns will be resolved, evaluated and classified as Ca Type or N Type (normal), the latter including normal, benign breast tumor, breast cancer in remission (post-surgical). After submission of evaluations, the data will be compared with the tumor classification to assess the correctness of diagnosis and to determine if other correlations can be made with relationship to nodal involvement, metastases, cell type, etc.

Progress: In excess of 950 blood samples have been collected and electrophoresed and densitometry completed. All samples are processed minimally as duplicate determinations utilizing 10 µl serum. Based on currently established criteria, preliminary diagnosis on 850 samples has been completed and correlated with the pathology diagnosis. These data indicate the following correct correlations: normals 79%; dysplasia, etc. 70%; fibroadenoma 74%; fibrocystic disease 68%; and pre-surgical carcinoma 70%. Sixty-seven postoperative serum samples were obtained in the acquisition group, 201-700. Of this number, 51 patients had no evidence of disease clinically and 42 patients were reported LDH negative; correlation value 83%. Of the remaining 16 clinically positive patients, 11 patients exhibited positive LDH patterns; correlation value 69%. Mammographic diagnosis is correlated at a level of 85%.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ronald B. Herberman, M.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 10/26/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $34,440

CONTRACT RESEARCH SUMMARY

Title: Steroid Excretion Study in Patients with Breast Cancer

Principal Investigator: Dr. Per Vestergaard
Name/Address
Performing Organization: Research Foundation for Mental Hygiene
Orangeburg, NY

Contract Number: NO1-CB-71465
Starting Date: 6/28/67 Expiration Date: 6/27/77

Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: The analysis of steroid hormones is based on column chromatography, the column consisting of fine, capillary Teflon tubings kept in a constant temperature bath. After the urine extracts are deposited at the head of the column, automation takes over so that all steps become highly reproducible. Even though the analysis per se is nonspecific, the high number of fractions collected allows for fine separation of metabolites without cross-contamination.

Progress: The special instrumentation developed under the contract has been described in a series of papers. A monograph giving full details of the methodology has been written and sent to a publisher. Some of the data from the patient studies have been submitted for publication; others are still being evaluated and prepared for publication.

The contract has been running on an extension basis without additional funding for several years.

Significance for Cancer Research (NCP Objective 6 Approach 6)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/28/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Pathology Laboratory for Cooperative Clinical Studies

Principal Investigator: Dr. Gilbert H. Friedell
Name/Address St. Vincent Hospital
Performing Organization: Worcester, MA

Contract Number: NO1-CB-80704
Starting Date: 3/18/68 Expiration Date: 4/17/78

Goal: To identify and define pathologic prognostic features in cases accessioned in the Primary Therapy of Breast Cancer Study; to study these features in comparable pathologic material from British, Japanese, and Hawaiian-Japanese breast cancer patients; and to correlate this information with clinical and other laboratory data from these cases.

Approach: The pathologic material collected at the Central Pathology Laboratory from the Collaborating Clinical Centers, and that subsequently received from British, Japanese, and Hawaiian-Japanese sources, has been--and is being--reviewed without knowledge of the clinical findings or other laboratory data, after which the correlations are carried out. Subsequent to the initial correlative efforts, the pathologic material is--and will be--reviewed again in an attempt to pinpoint findings of particular importance.

Progress: The tumor type and grade, nuclear grade, and presence of blood vessel invasion at the primary tumor site, and the presence of regional lymph node involvement have been determined in 381 cases and a report defining the study has been published. A report of some clinico-pathologic correlations was presented at the May 1977 ASCO meeting in Denver and a manuscript has been submitted for publication. A workshop on sinus histiocytosis utilizing material from the Central Pathology Laboratory was carried out and a report has been submitted for publication. A review of sinus histiocytosis in the 381 cases is in progress. A joint review with other pathologists of sinus histiocytosis in axillary lymph nodes from Japanese, Hawaiian-Japanese, and British breast cancer patients has been completed and a manuscript is in preparation. A detailed review of all axillary lymph nodes in radical mastectomy specimens from Japanese patients treated five or more years at the National Cancer Center Hospital in Tokyo is almost completed.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0 (No Cost Extension)

CONTRACT RESEARCH SUMMARY

Title: Biochemical Analysis of Human Breast Cyst Fluid (BCF)

Principal Investigator: Dr. Morton K. Schwartz
Name/Address
Performing Organization: Sloan-Kettering Institute for Cancer Research
New York, NY

Contract Number: NO1-CB-53853
Starting Date: 6/30/75 Expiration Date: 9/14/78

Goal: To establish the biochemical and immunochemical composition of human BCF; to search for a marker which might be useful in understanding the formation of cysts, their treatment, and the risk of patients with cysts to develop cancer.

Approach: BCF from patients with cystic mastopathy will be analyzed for polypeptide and steroid hormones, tumor-associated antigens, enzymes, lipids, minerals and trace elements. These data will be correlated with each other and with the demographic information. Fluid will be analyzed from patients with recurrent cysts and clinical follow-up will be obtained on all patients.

Progress: As of March 23, 1977, detailed analysis had been carried out on BCF from 589 patients. Seventy-two of these patients have had recurrent cysts and additional specimens submitted for analysis since their initial aspiration. As previously noted, the concentrations in many BCF specimens of CEA, α-fetoprotein, β-subunit hCG, calcium, copper, zinc, total protein, albumin, cholesterol, phosphohexose isomerase, γ-glutamyl transpeptidase, β-glucuronidase, lactic dehydrogenase, amylase, dehydroisoandrosterone, androsterose, and their sulfates are many fold the concentrations of these constituents found in serum. Correlation of BCF concentrations for many of these constituents between patients were found to be significant. The specimen variation was much less in specimens from the same patient obtained either at the same time or on subsequent aspiration than that from patient to patient. Notwithstanding the large BCF to serum gradient, isotope studies have indicated a slow influx and efflux of the steroids into the cyst fluid. The fluid appears to contain material both of a secretory nature and that resulting from cell breakdown.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D., R. Quentin Blackwell, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 5/5/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $98,060

CONTRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator: Howard H. Sussman, M.D.
Name/Address Stanford University Medical Center
Performing Organization: Stanford, California 94305

Contract Number: NO1-CB-74086
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To assay human breast cancer tissue and sera prior to mastectomy and throughout the clinical course of the disease for potential markers to determine how effective such markers would be for early detection of breast cancer and its recurrence and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Tumor and sera of patients presenting initially for diagnosis and primary therapy (category 1) will be assayed for the fetoplacental proteins, i.e. placental alkaline phosphatase (PAP), human chorionic gonadotropin (hCG) α and β, human placental lactogen (hPL) and carcinoembryonic antigen (CEA), and tests on sera will be repeated sequentially every three months. Patients developing recurrences (categories 2 and 3) will be included with a study population from the Northern California Oncology Group. Another objective of the study will be to identify new ectopically synthesized placental membrane proteins which may be common to both trophoblasts and neoplastic breast tumor cells.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/4/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $112,479

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator: Dr. Anne C. Carter
Name/Address State University of New York
Performing Organization: Brooklyn, NY

Contract Number: NO1-CB-23231
Starting Date: 4/16/72 Expiration Date: 12/14/77

Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for two years in regard to possible recurrence of the disease. An essential part of follow-up is the study of laboratory findings and x-rays taken for a minimum of two years to determine recurrences.

Progress: Fifty-six patients were entered on the study. Twenty patients recurred within two years. Twenty-nine patients have had no recurrence, 17 patients have died, and three patients have been lost to follow-up. Patients without recurrence are being followed at 6-month intervals until recurrence. Survival data are to be obtained on all patients.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Thor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 6/8/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Analysis of Breast Secretions

Principal Investigator: Dr. Nicholas L. Petrakis
Name/Address
Performing Organization: University of California School of Medicine
San Francisco, CA

Contract Number: NO1-CB-33882
Starting Date: 5/15/73 Expiration Date: 5/14/77

Goal: To evaluate Sartorius breast pump technique as a means of breast cancer detection, using cytological, biochemical and virological analysis.

Approach: Several studies are being pursued: (a) Correlation of cytologic findings on nipple aspirates in women with undiagnosed breast masses and comparison with mammography and surgical pathology. Attempts are being made to find tumor cells, cellular atypia, and/or specific biochemical changes. (b) Aspirations from women attending the ACS-NCI Breast Cancer Demonstration Center at Merritt Hospital in Oakland.

Progress: (a) A detailed analysis has been made of 3,758 breasts examined cytologically. Of 240 breasts which had been biopsied, the cytological findings were classed as severe dysplasia and as normal or hyperplasia:

	Cytological Diagnosis			
Pathological Dx	Severe Dysplasia	Normal or Hyperplasia	Total	% Severe
Breast cancer	14	30	44	31.8
Benign breast disease	3	192	196	1.5

$X^2 = 50$; $p < 0.001$

An additional 66/3,518 (1.9%) breasts had severe dysplasia but were otherwise normal clinically. These findings strongly support an association between severe cytological dysplasia and breast cancer. The ultimate outcome in women with severe dysplasia but with clinically normal breasts may provide an indication of their potential risk of breast cancer. (b) Severe atypia was found in fluid from 40/81 (49.4%) of women with a family history of breast cancer as compared to 80/231 (34.6%) of women without this history ($p<0.02$). These findings suggest that women with a positive family hsitory may be more prone to cytologic alterations.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/4/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Screening for Breast Cancer by Electronic Infrared Pattern Recognition

Principal Investigator: Dr. JoAnn D. Haberman
Name/Address University of Oklahoma Health
Performing Organization: Sciences Center
Oklahoma City, OK

Contract Number: NO1-CB-43869
Starting Date: 4/1/74 Expiration Date: 9/29/78

Goal: To design and clinically test a prototype system for mass screening of breast cancer utilizing quantitative thermography and computer processing with automated methods of evaluation.

Approach: This project is Phase 2 of a proposed 3-phase development program. The first phase included the following: (1) Construction of an improved thermographic instrument designed with the unique serial scan multiple element detector developed by Honeywell. This instrument provides radiometrically calibrated infrared imagery which is subsequently digitized. The instrument is equipped for direct recording on a standard video recorder and for conventional television display. (2) Initiation of a program of clinical evaluation to assess the utility of various quantitative parameters in selecting the thermographic changes of breast cancer. (3) A time-series evaluation of a group of subjects to obtain estimates of the influence of several physiological factors on the thermal patterns of the breast. Phase 2 consists of further clinical evaluation with digitization of thermal images and analysis by a variety of pattern recognition schemes for feature development and classifier design and construction.

Progress: Clinical evaluations are proceeding on groups of symptomatic and asymptomatic subjects. The early months of Phase 2 were devoted to system improvement with the addition of a 6 Bit A/D conversion video digitizer which provides "on-line" digitization of the thermal image. Advantages of an "on-line" system include eliminating noise as well as possible systematic errors in transfer of analog information. Presently, all thermograms are digitized "on-line" and during the period of system modification, absolute temperatures were manually recorded.

To date, digitized thermograms have been collected on 2,256 patients, including 55 cancers and 151 benign biopsies. Breast temperature measurements are being examined to determine their discrimination ability of cancer and control subjects. Data collected on the cancer cases are encouraging and the blood perfusion estimate statistic appears to be the best discriminator. Ten thousand patients and 150 cancers will comprise the data base of Phase 2 of this program.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/1/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $336,514

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator: Dr. Carleton R. Haines
Name/Address University of Vermont
Performing Organization: Burlington, VT

Contract Number: NO1-CB-23232
Starting Date: 3/13/72 Expiration Date: 9/11/77

Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for two years in regard to possible recurrence of the disease. An essential part of follow-up is the study of laboratory findings and x-rays taken for a minimum of two years to determine recurrence.

Progress: Forty-six patients have been entered since June 15, 1972. Nine of these cases were negative for malignancy, while six were dropped for other reasons. Periodic follow-up examinations and reports at 3- and 6-month intervals were carried out as specified in the protocol. After two years patients are being followed and evaluated at 6-month intervals. In view of the relationship between some of the clinical and pathological factors and the rate of recurrence at two years, a longer period of follow-up has been recommended. Survival data are to be obtained on all patients.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 6/9/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Fibrocystic Disease and Its Relationship to Breast Cancer

Principal Investigator: Dr. David L. Page
Name/Address: Vanderbilt University
Performing Organization: Nashville, TN

Contract Number: NO1-CB-43871
Starting Date: 1/15/74 Expiration Date: 6/14/77

Goal: To determine which lesion, or combination of lesions, in chronic cystic mastopathy is associated with an elevated risk of subsequent mammary carcinoma development.

Approach: The investigators have assembled and classified slides from breast biopsies of 1,127 women performed 15 or more years before follow-up. All slides were classified as to the component parts of cystic disease, following a modification of the Foote and Stewart classification system, by two separate observers employing an analysis of interobserver variation.

Progress: Follow-up is complete on 1,058 women. After exclusion of women with *in situ* carcinoma on review of the entry biopsy (7 patients), patients with bilateral simple mastectomy and patients with breast cancer (infiltrative) at or before the entry biopsy, 966 women are left for analysis. The lesion most predictive of elevated carcinoma risk is atypical lobular hyperplasia which is associated with a greater than 3 times elevated risk over an age-matched group of women from the Third National Cancer Survey incidence figures for carcinoma of the breast in Atlanta. Various ductal hyperplastic lesions are associated with approximately a doubly increased risk which is significant only if identified at biopsy after the age of 45. Cysts by themselves and sclerosing adenosis are not associated with an elevated risk of subsequent carcinoma development.

Significance for Cancer Research (NCP Objective 5 Approach 5.2)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/13/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Risk Associated with In-Situ Carcinoma and "Precancerous" Mammary Hyperplasias

Principal Investigator: David L. Page, M.D.
Name/Address Vanderbilt University School of Medicine
Performing Organization: Nashville, Tennessee 37232

Contract Number: NO1-CB-74098
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To compare subgroups of females regarding their incidence of breast carcinoma during a 10 to 25 year interval after biopsy diagnosis of in situ carcinoma and mammary hyperplasias.

Approach: This will be a retrospective study in which slides will be reviewed and classified on over 4000 patients who had biopsies performed during 1952-1969. This study population will then be contacted and follow-up information on each subject obtained regarding subsequent development of mammary carcinoma, as well as other pertinent clinical and epidemiological data. The control group will be composed of patients with biopsy but no evidence of proliferative lesions. An effort will be made to identify those specific breast lesions or combinations of lesions which are associated with an increased risk of development of breast cancer. Non-anatomic risk factors will also be correlated.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 4/13/77
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: Approx. $112,000

CONTRACT RESEARCH SUMMARY

Title: Blood Steroid Sulfate Levels as Diagnostic Parameters in Breast Cancer

Principal Investigator: Dr. Federico Welsch
Name/Address Worcester Foundation for Experimental
Performing Organization: Biology
Shrewsbury, MA

Contract Number: NO1-CB-43898
Starting Date: 1/1/74 Expiration Date: 12/31/76

Goal: To develop assays for estrone sulfate (E_1S), androsterone sulfate (AS), and dehydroepiandrosterone sulfate (DHAS) so that we can analyze blood steroid sulfate levels to serve as possible diagnostic and/or prognostic parameters in breast cancer.

Approach: Estrone sulfate, dehydroepiandrosterone sulfate, and androsterone sulfate will be measured in the plasma of patients either with diagnosed breast cancer or diagnosed benign breast tumors or apparently normal controls using radioimmunoassay techniques. These measurements can be used to indicate possible changes in adrenal steroid production in postmenopausal women and adrenal and ovarian activity in young women occurring after the development of breast cancer.

Progress: Previous methodology has been reviewed and revamped so that ^3H-E_1S, ^3H-AS and ^3H-DHAS are added to all plasma samples to monitor recoveries; solvolysis is carried out on all sulfates using 0.01% H_2SO_4 in ethyl acetate.

With these modifications the inter- and intra-assay variations are 6-12% for all steroids. The addition of steroid glucuronides does not change the values; when plasma from adrenalectomized-ovariectomized subjects are analyzed, the values for E_1S, AS and DHAS do not differ from the water blank. Mean $\pm$ SE values in normal subjects are as follows:

	n	E_1S pg/ml	AS µg/ml	DHAS µg/ml
men	14	358 $\pm$ 38	0.50 $\pm$ 0.11	1.10 $\pm$ 0.19
women, days 5-7	10	726 $\pm$ 133	0.49 $\pm$ 0.06	0.97 $\pm$ 0.17
" , " 20-22	10	965 $\pm$ 148	0.48 $\pm$ 0.09	1.29 $\pm$ 0.25
postmenopausal women	13	150 $\pm$ 12	0.12 $\pm$ 0.03	0.38 $\pm$ 0.07

The postmenopausal women have significantly ($p<0.01$) lower values than both men and young women for all steroid sulfates.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Ihor J. Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/4/76
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Estrogen Replacement after Pre-Menopausal Oophorectomy and Breast Cancer Risk

Principal Investigator: Dennis Slone, M.D.; Samuel Shapiro, M.D.
Name/Address Drug Epidemiology Unit
Performing Organization: Boston University Medical Center
Cambridge, Massachusetts 02138

Contract Number: NO1-CB-74099
Starting Date: 8/77 Expiration Date: 7/78

Goal: To study estrogen replacement therapy following pre-menopausal oophorectomy in relation to risk of subsequent breast cancer.

Approach: The study will be part of a large, multiple case-control study aimed at monitoring long-term ambulatory use of drugs in general, as it relates to the risk of serious morbidity. Nurse-interviewers will be stationed in selected hospitals throughout the United States and abroad, and will obtain from hospital patients with selected diagnoses a large amount of descriptive medical data, together with comprehensive histories concerning prior drug use. At least 500 breast cancer cases will be drawn from these hospitals and their histories compared with those of 2000 age-matched hospital controls, about half women with newly diagnosed "non-female cancers" and half women with acute non-malignant conditions requiring hospitalization. Efforts will be made to validate histories of oophorectomy and estrogen replacement therapy for at least a sample of the subjects. The data collection will allow for a latent period of 15 years or more in breast cancer development. Sample size will be increased if deemed necessary.

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 2/25/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $37,000

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Dietary Factors and Pre- and Postmenopausal Breast Cancer

Principal Investigator: Dr. Baruch Modan
Name/Address Chaim Sheba Medical Center and
Performing Organization: Tel Aviv University Medical School
Tel Hashomer, Israel

Contract Number: NO1-CB-53883
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To carry out epidemiological investigations of selected population groups to elucidate the possible role of diet and/or nutritional status on the incidence, pathogenesis, and natural history of pre- and postmenopausal breast cancer.

Approach: A case-control study using a validated questionnaire is being conducted to investigate the following possible risk factors for pre- and postmenopausal breast cancer: dietary components; weight and height; radiotherapy treatment; breast feeding; long-time drug use; personal and family history of breast disease. The study population is composed of 720 newly diagnosed breast cancer cases (pre- and postmenopausal) of which 450 will be European and American born, 200 will be Asian-African born, and 70 will be Israeli born. There are two control groups: one drawn from hospitals, one from neighborhoods. These are matched to the cases for age, country of origin, and period of immigration to Israel. All analyses will be performed separately for ethnic groups, histopathologic category, and by menopausal status.

Progress: As of May 31, 1977, a total number of 1,460 hospital and home interviews have been completed. These include 528 cases, 483 surgical controls, and 449 neighborhood controls. Previous history of breast disease and other surgical conditions is being validated by reviewing hospital records. Preliminary computer editing programs were prepared. Coding and checking have been completed on approximately two-thirds of the questionnaires. A histopathological classification has been prepared.

Significance for Cancer Research (NCP Objective 5 Approach 5.1 and 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/8-9/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $51,141

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator: Baruch Modan, M.D.
Name/Address Chaim Sheba Medical Center, Tel Hashomer
Performing Organization: and Tel-Aviv University Medical School
Tel Hashomer, Israel

Contract Number: NO1-CB-74102
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To establish the epidemiologic features of benign breast diseases and to determine their relevance to the established risk factors for breast cancer.

Approach: The proposal will consist of two phases. The first will be a case control interview study of 600 cases of non-invasive breast disease, in terms of such risk factors as age at first birth, menstrual history, menopausal status, parity, height and weight, familial breast cancer, selected drug history, dietary intake, and previous radiation exposure. The findings will be compared with their similarly conducted breast cancer study. The second phase will be a nation-wide study of the incidence of non-invasive breast lesions. In both phases, particular attention will be paid to the relative frequency of distinct histo-pathological forms in high and low risk sub-populations for breast cancer (i.e. European vs. Asian and African born). The characterization of the breast lesions will take account of the histological pattern, degree of atypia,and lymphoreticulo-endothelial response (LRE).

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $51,543

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Family Resource

Principal Investigator: Dr. Henry T. Lynch
Name/Address Creighton University School of Medicine
Performing Organization: Omaha, NE

Contract Number: NO1-CB-33901
Starting Date: 6/30/73 Expiration Date: 6/29/77

Goal: To analyze data on the 87 breast cancer-prone families which now comprise the resource. Such an analysis includes risk factors determined for cancer-proneness in these families.

Approach: The resource continues to supply sample acquisitions to various investigators for the following studies: (1) human lymphocyte-antigen (HL-A); (2) carcinoembryonic antigen studies; (3) bovine C-type virus; (4) viral antibody and cytogenetic studies; (5) genetic polymorphisms; (6) aryl-hydrocarbon hydroxylase; (7) virology studies on placentas, tumor specimens and leukocytes using electron microscopy; (8) mammalian C-type RNA tumor viruses; (9) SMT-antigen; (10) Type-C viruses; (11) lymphocyte cytotoxicity; (12) immunological studies including T and B lymphocytes and lymphocyte alpha-fetoprotein; and (13) DNA repair. Efficient and meaningful use of genealogic information from the breast cancer resource involves sophisticated utilization of a highly developed computer system.

Progress: A total of 85 pedigrees meet our selection criteria for a breast cancer family (at least 2 first degree relatives with breast cancer). The total resource contains information on approximately 2,200 direct line females and 1,800 direct line males. There are 408 female cancer patients (302 with breast cancer) and 155 male cancer patients. More than 1,000 direct line females, many of whom are at high genetic risk for breast cancer, are available for ascertainment of biological specimens. The estimate of the lifetime breast cancer risk to daughters of affected mothers is in excess of 40%. Biological specimens obtained from high-risk individuals are being supplied to various investigators for the following studies: (1) human lymphocyte-antigen (HL-A); (2) carcinoembryonic antigen (CEA); (3) bovine C-type virus; (4) viral antibody and cytogenetic studies; (5) genetic polymorphisms; (6) aryl-hydrocarbon hydroxylase; (7) virology studies on placentas, tumor specimens and leukocytes using electron microscopy; (8) mammalian C-type RNA tumor viruses; (9) SMT-antigen; (10) type-C viruses; (11) lymphocyte cytotoxicity; (12) immunological studies including T and B lymphocytes and lymphocyte alpha--fetoprotein.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 2/3/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High- and Low-Risk Breast Cancer Families

Principal Investigator: Dr. Henry T. Lynch
Name/Address Creighton University School of Medicine
Performing Organization: Omaha, NE

Contract Number: NO1-CB-44003
Starting Date: 6/17/74 Expiration Date: 6/16/78

Goal: To investigate histocompatibility antigens and other genetic polymorphisms in high and low risk breast cancer families.

Approach: HL-A markers, genetic polymorphic markers such as red blood cell enzymes (acid phosphatase, adenosine deaminase, etc.) serum proteins (haptoglobins a-chain, group-specific component, etc.), and blood group systems (ABO, Duffy, Lewis, MN, etc.), and specific pathologic diagnoses (all histologic varieties of cancer retrospectively in the family context) are being evaluated in approximately 20 multiple cancer case families selected from the total clinical pool of high risk families and kindreds. Segregation and linkage analyses of pedigrees will be performed, and quantitative biomarkers will be incorporated into an index of breast cancer susceptibility utilizing general methods for deriving selection indices.

Progress: Twenty-one case families have been sampled. A total of 720 individuals have been seen and blood samples obtained. Specimens have been sent to the investigators. HL-A data has been analyzed for seven of the breast cancer-prone families, and other markers have been analyzed for nine families categorized as at highest risk. Linkage analysis is now being undertaken. Eighty-five pedigrees appear suitable for segregation analysis and 18 of these will be subjected to linkage analysis contingent upon confirmation of qualitative inheritance. The resource continues to show genetic heterogeneity as evidenced by at least 5 distinct tumor entities. Twelve pedigrees show predisposition to breast/ovarian carcinomas with an approximate 50% lifetime risk to daughters of affected mothers. An even higher rate of transmission from affected paternal grandmothers leaves open the possibility of X-linkage in these particular pedigrees.

Significance for Cancer Research (NCP Objective 5 Approach 5.14)

Project Officer: Joseph Fraumeni, M.D., Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 2/3/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $20,000

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator: Dr. David C. Deubner
Name/Address
Performing Organization: Duke University Medical Center
Durham, NC

Contract Number: NO1-CB-63996
Starting Date: 6/29/76 Expiration Date: 6/29/78

Goal: The goal is to define the epidemiologic characteristics of breast cancer in pre- and postmenopausal women and to relate these characteristics to the pathobiology of the tumor as manifested by estradiol and progesterone receptors, histology, and cellular ultrastructure.

Approach: This case-referent study is of breast cancer patients undergoing surgery at university and community hospitals, surgical or medical patients not having breast or gynecological surgery at the same hospitals, and subjects from the community. Data collection is by interview of all subjects and review of charts of hospital patients. Laboratory studies on tissue and serum from breast cancer patients will include quantitative estrogen and progesterone receptor determinations, evaluation of histology and ultrastructure by a standardized protocol, and estrogen and progesterone serum determinations. Statistical analyses will estimate the relative strength of association between breast cancer in pre- and postmenopausal women and such factors as age of menarche, age at first delivery, obesity, and family history of breast cancer. Associations between risk factors and tumor cell biology and morphology will be sought by comparing the laboratory results in women with and without single or multiple risk factors.

Progress: Effective working relations have been established with the clinicians and pathologists in the three participating hospitals. As of May 25, 1977, 160 women with breast cancer had been entered in the study and satisfactory interviews and pathologic materials obtained from at least 85%. Two hospital referent subjects, matched by race and age, have been interviewed for each case entered. The community referent group is a random sample of women at least 30 years of age who live in the piedmont and plains regions of North Carolina. Approximately 200 of the anticipated 1,800 interviews have been completed since the field work began in March 1977. Laboratory procedures are being completed according to protocol as material is obtained from the participating pathologists.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Gordon B. Cutler, M.D.
Program: Breast Cancer Epidemiology Site Visit Date: 5/5/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $174,961

CONTRACT RESEARCH SUMMARY

Title: Endocrine Events at the Time of First Pregnancy

Principal Investigator: John R. K. Preedy, M.D.
Name/Address Emory University School of Medicine
Performing Organization: Atlanta, Georgia 30303

Contract Number: NO1-CB-74101
Starting Date: 8/77 Expiration Date: 7/78

Goal: To study endocrine events at the time of first pregnancy, in relation to age, relevant to the protection by early first pregnancy against subsequent development of breast cancer.

Approach: The hormonal environment of normal subjects will be investigated before and after the first pregnancy, to determine whether any consistent and identifiable permanent or semi-permanent change has taken place in hormone levels, or in the ratio of one hormone to another. Groups of subjects with a first pregnancy before the age of 20 and after the age of 30-35 will be studied to ascertain whether any changes so identified are related to the age of first pregnancy. Half of each group will be drawn from a black and half from a white population. The women will be studied before conception for the first pregnancy, and again three months to one year after completion of their first full-term pregnancy plus any period of lactation. Studies will be made over a similar time period on comparable women who have not undergone pregnancy. The studies will estimate a number of hormones in blood and urine, with and without stimulation and suppression procedures. These will include a number of estrogens and estrogens conjugates, a number of androgens, progesterone, prolactin, follicle-stimulating hormone and luteinizing hormone; phase of the menstrual cycle to be used for sampling will be standardized.

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 6/6/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $115,000

CONTRACT RESEARCH SUMMARY

Title: Pathogenetic Models of Malignant and Pre-Malignant Disease of the Breast

Principal Investigator: J.A.H. Lee, M.D., D.P.H.; Suresh Moolgavkar, M.D., Ph.D.
Name/Address Fred Hutchinson Cancer Research Center
Performing Organization: Seattle, Washington 98104

Contract Number: NO1-CB-74100
Starting Date: 8/77 Expiration Date: 7/78

Goal: To develop a coherent hypothesis, in mathematical terms, of the pathogenesis of breast cancer that is sufficiently explicit to be rigorously tested against current data, and which can lead to testable predictions.

Approach: The hypothesis is a variant of the multistage model for carcinogenesis. It conjectures that a normal cell must pass through several stages before being transformed into a malignant cell. At each of these intermediate stages, the cell could be repaired or destroyed by the body. The model proposes that the malignant state itself consists of two sub-states and that the female sex hormones exert their influence at this stage. The study will be divided into three phases: (1) Solution of the equations generated by the model; study of these solution under perturbations of the rate constants. (2) Use of data already available in cancer registries to test the model. (3) Fitting of computer-generated incidence curves predicted by the model to data from different populations. The three phases will proceed concurrently and information obtained will be used to study the rate constants in the model and to modify the model as required by the relationship between its predictions and actual data. The model will also be capable of progressive modification by new data.

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/25/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $80,000

CONTRACT RESEARCH SUMMARY

Title: A Study on Familiality of Breast Cancer in Iceland

Principal Investigator: Dr. Nicholas E. Day
Name/Address (Dr. H. Tulinius: Co-investigator in Iceland)
Performing Organization: International Agency for Research on Cancer
Lyon, France

Contract Number: NO1-CB-43973
Starting Date: 6/30/73 Expiration Date: 9/29/77

Goal: To determine whether there are hereditary characteristics which might be of use in the identification of women having a high risk of developing breast cancer.

Approach: The unique facilities available in Iceland for the study will be utilized. These include (1) a nationwide cancer registry functioning since 1954 (about 800 cases), all cases of breast cancer diagnosed between 1910 and the start of the cancer registry are on file; (2) a computer-based National Roster since 1952; (3) a family linkage file linking every individual alive after 1910 to his parents and children; and (4) a cervical screening clinic which has seen more than 80% of the female population of Iceland born between 1905 and 1945. Information is collected on reproductive history and age at menarche, to be used as control information. If an excess risk is found, an effort will be made to (1) correlate familial risk with recognized epidemiological risk factors for breast cancer, (2) quantify the risk attached to different types of relationships, and (3) for each class of relative, examine the association, if any, between the degree of risk and the age of the proband case and age of appearance of cancer among these relatives.

Progress: Age, year of birth, and age at first live pregnancy have independent multiplicative effects on the risk that a woman develops breast cancer in Iceland. The relevant parameters have been estimated, resulting in a risk function applicable to all Icelandic women. Genealogies of 419 families have been completed and computer linked to the file of cases. A number of doubtful matches are being verified by the interviewers in Iceland. Papers for publication have been prepared on the effect of reproductive factors on risk for breast cancer in Iceland and on statistical methods for parametrising disease clustering. A paper on familial risk in Iceland is also under way.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $35,000

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Morphology and Epidemiology of Breast Cancer

Principal Investigator:	Dr. H. Stephen Gallager
Name/Address	University of Texas System Cancer Center
Performing Organization:	M.D. Anderson Hospital and Tumor Institute
	Houston, TX

Contract Number: NO1-CB-53854
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To demonstrate morphologic markers characteristic of familial types of breast disease in order to identify selected high-risk populations.

Approach: A previously developed system for the profiling of the morphologic characteristics of breast cancer which integrates information from gross and microscopic examination, mammography, and specimen radiography and requires the rough quantitative evaluation of some 39 factors in three groups will be validated and refined. The breast cancer specimens will be derived from three major risk groups which have been characterized genetically. These consist of 114 patients with mothers with cancer pedigrees, 97 patients with sisters with cancer pedigrees, and 111 patients with second order pedigrees for breast cancer. The findings will be compared with 300 patients without family histories.

Progress: The planned validation phase of the study has been completed and modifications of the data collection system and evaluative criteria have been completed. A paper is in preparation concerning the biomathematical techniques used in this phase. It is believed that they may have relevance to the development of quality control procedures for diagnostic histopathology. General data recording using the refined system began about December 1, 1975. To date, approximately 215 cases have been completed by all three observers and an additional 30 cases have been completed by one or two observers.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D., Philip Prorok, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/28/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $40,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical and Physiological Investigations Based on Familial Genetic Patterns

Principal Investigator: Dr. Jack Fishman
Name/Address Montefiore Hospital and Medical Center
Performing Organization: Bronx, NY

Contract Number: NO1-CB-44002
Starting Date: 6/24/74 Expiration Date: 6/23/77

Goal: To evaluate the possible role of genetic factors in the expression of specific biochemical parameters in breast cancer pathophysiology.

Approach: Endocrine assays will be done on blood and urine of premenopausal females from families with a high incidence of breast cancer and from matched control families. Morning blood samples obtained every second day throughout the menstrual cycle will be assayed for FSH, LH, E_2, E_1, progesterone and prolactin. Estriol will be measured on pooled follicular and luteal plasma samples. Nocturnal urines (7 p.m. to 7 a.m.) will be assayed for 13 estrogenic, androgenic and corticosteroid metabolites. An attempt will be made to correlate familial susceptibility to breast cancer with endocrine parameters.

Progress: This report covers the period ending May 31, 1977. Plasma and urine samples from 60 women have been received. Plasma hormone measurements are now completed in all 60 subjects. Except for 2-methoxyestrone, urinary steroid measurements are complete in all 60 subjects. 2-Methoxyestrone has been assayed in 27 subjects on pooled follicular and luteal urinary extracts. Preliminary evaluations by computer analysis have afforded the following results. Urinary excretion of estrone is significantly lower in the high-risk group. The estriol proportion ($E_3/E_1 + E_2 + E_3$) is significantly higher in the high-risk group. Urinary excretion of both dehydroisoandrosterone sulfate and glucuronide are higher in the high-risk group. The ratio of urinary cortisol metabolites (THF + THE + ATHF) to urinary DHAG + DHAS is lower in the high-risk group. The cortisol metabolites showed no significant difference between the two risk groups.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D
Program: Breast Cancer Epidemiology Site Visit Date: 9/27/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $30,000

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High- and Low-Risk Breast Cancer Families

Principal Investigator: Dr. Frans J. Cleton
Name/Address The Netherlands Cancer Institute
Performing Organization: Amsterdam, The Netherlands

Contract Number: NO1-CB-43940
Starting Date: 6/18/74 Expiration Date: 6/18/78

Goal: To study linkage of certain genetic markers with breast cancer in an attempt to select high-risk groups in the population for the development of breast cancer.

Approach: HLA haplotypes will be studied in families with a high incidence of breast cancer. Certain genetic markers located on the 6th chromosome and the red blood cell groups will also be investigated. The study of red cell isoenzymes, immunoglobulins, and chromosomal polymorphisms have been stopped after the analysis of data on 14 families. The search for cross-reacting antigens with mouse MTV-sera, yielding negative results, has also been stopped. Plasma-prolactin levels will be measured in breast cancer patients and their daughters, with special emphasis on diurnal fluctuation and variation during the menstrual cycle. Because multiple case families with sufficient living affected members in two generations are rare, it was decided also to investigate pairs of sisters with breast cancer. Linkage of breast cancer with HLA can be determined by comparing similarities in HLA haplotypes in these pairs with the expected similarity.

Progress: Linkage studies have been carried out on 700 members from 14 high-risk families, including 60 patients with breast cancer. There was no significant evidence of linkage between breast cancer and any of the 70 genetic markers that were studied. A computer program for linkage analysis, especially designed for incomplete family data, was used and the results were expressed as lod scores. Because the sample may have been too small for this method of analysis, more high-risk families will be investigated. New methods for analyzing the results of the family studies will be used. The sample will be increased with similar data from the investigation of two other contractors. Ten sister pairs have been typed for HLA in addition to the family studies. Studies of prolactin in healthy female relatives in these families showed an irregular distribution of plasma levels, probably related to an altered diurnal regulation. These studies will be extended, including the variation of plasma prolactin during the menstrual cycle.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Joseph Fraumeni, M.D., Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Relation of Intestinal Flora to Breast Cancer in Humans

Principal Investigator: Sherwood Gorbach, M.D.
Name/Address New England Medical Center
Performing Organization: Boston, Massachusetts 02111

Contract Number: NO1-CB-74104
Starting Date: 7/1/77 Expiration Date: 6/30/78

Goal: To determine the role of the intestinal microflora in the metabolism of biologically significant estrogens in women who are at high and low risk for breast cancer.

Approach: The study will aim to identify and measure the biologically significant estrogens in women consuming diets that predispose to low and high risk of breast cancer. There will be four groups of 10 women each: of age 20-25 and age 55-60 on a Western diet (high risk) and of equivalent ages on a vegetarian diet (low risk). A fifth group of breast cancer cases, age 55-60, on a Western diet, will also be studied. Each woman will be studied a total of four times at 16-week intervals. The metabolic activity of the intestinal flora will be examined in terms of capacity to metabolize steroids and possibly alter estrogen metabolism.

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/13/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee

FY 77 Funds: $97,000

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Minimal Breast Cancer in Initially Asymptomatic Women

Principal Investigator: Bernard Pasternack, Ph.D.
Name/Address New York University Medical Center
Performing Organization: New York, New York 10016

Contract Number: NO1-CB-74103
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To investigate by risk factor analysis whether minimal breast cancer in initially asymptomatic women is different from clinical breast cancer or is an early manifestation of the same disease.

Approach: The study will compare risk factors for minimal breast cancer with known risk factors for breast cancer. Minimal lesions will be defined as 1.0 cm. or less and will be classified by histologic type and by level of invasiveness and aggressiveness. The project will also aim to compare prevalent lesions with incident lesions in order to identify risk factors associated with tumor aggressiveness. The data to be analyzed will be those collected since 1968 by the Guttman Breast Diagnostic Institute. Risk factors to be examined are age, race, religion, socioeconomic status, age at menarche and at first birth, parity and lactation, menopause, if reached, including artificial menopause, obesity, exogenous estrogens, and history and family history of breast disease.

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 5.2 and 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 2/17/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $111,300

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Medullary and Lobular Breast Cancer

Principal Investigator: Dr. Paul P. Rosen
Name/Address Sloan-Kettering Institute for Cancer
Performing Organization: Research
New York, NY

Contract Number: NO1-CB-63997
Starting Date: 6/29/76 Expiration Date: 6/29/78

Goal: To identify and describe morphologic features of human female mammary carcinoma which are significantly related to known risk factors for the development of such carcinomas.

Approach: Detailed epidemiologic information will be obtained by interview from women with mammary carcinoma and correlated with pathologic features of the tumors. The analysis of the data will emphasize comparison of medullary and lobular carcinoma with duct carcinoma but will also permit the study of morphologic features not limited to one tumor type.

Progress: Four hundred fifty consecutively treated patients have been entered into the study. Among cases already reviewed are 22 women whose only lesion was medullary carcinoma, 13 with only in situ lobular carcinoma, and 9 with only infiltrating lobular carcinoma. Women who had mixed tumor patterns that included medullary or lobular carcinoma are not included in these figures. Interviews have been obtained with 97% of the patients. A program of data verification is being carried out for the information obtained by interview which related to prior medical or surgical therapy. Programs for entry of data for computer storage and analysis have been completed and entry of data thus far obtained has begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D., Mr. Roger Connelly
Program: Breast Cancer Epidemiology Site Visit Date: 4/21/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Morphology and Epidemiology of Breast Cancer

Principal Investigator: Dr. Bjorn Stenkvist
Name/Address University Hospital
Performing Organization: Uppsala, Sweden

Contract Number: NO1-CB-53968
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To ascertain possible correlations between morphologic variables such as cytologic and histopathologic classifications and established epidemiological risk factors of breast cancer.

Approach: Within a clearly defined area and population (4 Swedish counties) 181 breast cancer cases occurred during a defined study period (5 months). From 179 of these patients a number of epidemiological data could be obtained as well as morphological data. From 179 exactly age-matched controls epidemiologic data were recorded in the same way as the patient data. The tumors were also characterized by computerized photometry of the cell populations in the tumors in addition to conventional morphology. In the first phase of the epidemiological study breast cancer was regarded as an entity (i.e. not morphologically subdivided) and the patients were compared with their exactly age-matched controls.

Progress: The comparison between patients and controls showed no significant difference in age at menarche, age at first birth, age at menopause, number of children, height, weight, obesity or frequency of diabetes and hypertension. These results are at variance with most earlier reports, possibly because the controls here were selected from the whole female population instead of hospitalized patients. Heredity and frequency of earlier breast disease were significantly higher in the patient group. The occurrence of axillary metastases at the time of diagnosis was clearly related to the computer grading combined with a measure of tumor size. By such a combined measure the patients could be subdivided into two categories - one, low-risk category,with 15% metastases at the time of diagnosis and one, high-risk category, with 65% of the patients having metastases at the time of diagnosis.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Elizabeth P. Anderson, Ph.D., Cecil Fox, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/17/75
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $110,525

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator: Dr. Ralph S. Paffenbarger
Name/Address University of California
Performing Organization: Berkeley, CA

Contract Number: NO1-CB-63998
Starting Date: 6/29/76 Expiration Date: 6/29/78

Goal: This work would identify host and environmental characteristics that predispose to the development of breast cancer in women of all ages, and would contrast the findings between premenopausal and postmenopausal women.

Approach: Using epidemiologic methods of case-control design, we would conduct structured interviews with women in the San Francisco Bay area, totaling over 2,500 breast cancer patients and 5,000 control women. Characteristics to receive special attention as potential precursors of breast cancer would include age, catamenial and childbearing events, indicators of early fertility, exogenous hormone and drug use, sociocultural factors, family history of cancer, radiation exposure, and various physical measurements. Extent of disease (staging), histological diagnoses, and survival determinations would be established. Amassed data would be processed using appropriate statistical methods in search for associations between epidemiologic characteristics and breast cancer, premenopausal vs. postmenopausal.

Progress: Interviews have been completed on some 3,100 study subjects, one-third of whom are breast cancer patients of all ages and two-thirds of whom are age-, race-, and hospital-matched control patients. About 20% of the breast cancer patients are premenopausal. Nearly 2,900 interviews have been coded and keypunched for data processing. A series of computer-based editing filters have been programmed to check the validity and consistency of the data. Procedures to re-code and update edited records have been instituted, and the editing process is under way. A general computer program has been constructed to perform summary chi square analyses with adjustment for confounding factors, starting from primary data and user-prepared control cards. Documentation of the program is being prepared. Histological diagnoses have been made on about 400 breast cancer patients; these data are being coded and key-punched. Survival follow-up of breast cancer patients has been completed through December 1976. Preliminary analyses have been made of the risk of breast cancer as it depends upon ever-use of oral contraceptives, total duration of oral contraceptive use, and use of oral contraceptives before first childbirth.

Significance for Cancer Research (NCP Objective 5 Approach 5.1 and 5.6)

Project Officer: Elizabeth P. Anderson, Ph.D., Bruce Stadel, M.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/15/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $81,720

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator: Gary Spivey, M.D.
Name/Address University of California, Los Angeles
Performing Organization: Los Angeles, California 90024

Contract Number: NO1-CB-74202
Starting Date: 8/77 Expiration Date: 7/78

Goal: To establish the epidemiologic features of benign breast diseases and to determine their relevance to the established risk factors for breast cancer.

Approach: The epidemiology of major types of benign breast disease will be examined in a case-control study of 1000 women with benign breast disease, and 1200 controls in Los Angeles County. There will also be a comparison group of 200 breast cancer cases. Data will be collected on risk factors known to be relevant to breast cancer. These will include age at menarche, parity, age at first pregnancy, age at menopause, if reached, menstrual and reproductive history, including history of breast development and of artificial menopause, height, weight, measures of socio-economic status, family history of breast cancer, and history and family history of benign breast disease. A reproducible classification of types of benign breast disease is to be demonstrated and utilized, and is to include standard pathologic diagnosis as well as a system for accurate grading of atypical changes. Epidemiologic characteristics of women classified into the histologic subgroups will be compared.

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 5.2)

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 2/28/77
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $150,000

CONTRACT RESEARCH SUMMARY

Title: Role of Dietary Factors and Non-Contraceptive Estrogens in Breast Cancer

Principal Investigator: Dr. Abraham M.Y. Nomura
Name/Address University of Hawaii at Manoa
Performing Organization: Cancer Center of Hawaii
Honolulu, HI

Contract Number: NO1-CB-53884
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To determine in postmenopausal women if there is an association of breast cancer with specific dietary factors and the use of menopausal estrogens.

Approach: A case-control approach is being utilized in which a questionnaire is employed to collect information on past history of drug usage (including menopausal estrogens) and usual weekly dietary intake. Breast cancer cases from three selected populations (ages 45-74) are identified: (1) the Caucasians in Hawaii who are at high risk for breast cancer; (2) the Japanese in Hawaii who are at intermediate risk; and (3) the Japanese in Fukuoka, Japan, representing a low-risk population. For each case, a neighborhood and hospital control, matched by race and age, is interviewed. Attempts are made to verify the drug history with the subject's personal physicians and a subsample of the study population receives a repeated dietary interview to assess its reliability.

Progress: As of May 6, 1977, interviews in Hawaii have been completed on 124 cases, 112 hospital controls, and 102 neighborhood controls. In Japan, 72 cases, 68 hospital controls, and 70 neighborhood controls were interviewed, as of November 1, 1976.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Elizabeth P. Anderson, Ph.D.; David Levin, M.D.
Program: Breast Cancer Epidemiology Site Visit Date: 5/19/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High- and Low-Risk Breast Cancer Families

Principal Investigator: Dr. David E. Anderson
Name/Address University of Texas System Cancer Center
Performing Organization: Houston, TX

Contract Number: NO1-CB-44004
Starting Date: 6/16/74 Expiration Date: 6/30/77

Goal: To investigate a series of polymorphic genetic markers in high-, moderate-, and low-risk breast cancer families which might assist in the definition of high-risk individuals.

Approach: The ABO, Rh, MNS, P, K, Fy^a and Jk^a blood group types; serum haptoglobin type; acid phosphatase and phosphoglucomutase 1 red blood enzyme types; and chromosome abnormalities and/or variants and sister chromatid exchanges were determined from blood specimens collected from patients belonging to the three pedigree groups and from healthy control women. The results for each of the pedigree groups were compared with the controls and with one another for evidence of any association between a marker and breast cancer occurrence. The chromosome studies were restricted to patients and sisters from pedigrees in which the genetic effect was maximal as reflected by the occurrence of the disease in at least three first-degree relatives.

Progress: The markers were determined on 264 familial patients and 311 controls, but evaluation of the ABO system involved 497 patients and 1,226 controls. Information on the ABO system was also available on 1,887 unselected patients and 626 blood donor controls. Of the genetic markers, only the ABO system showed an association with breast cancer. The frequency of type A was in excess and type O was deficient in all pedigree groups, but notably so in the moderate and low-risk groups compared with controls. A similar difference applied to the unselected patients compared with their controls. Age of the patient had an important effect on the ABO distribution. Type A increased and type O decreased significantly with an increase in age. No age effect was observed in the controls nor for the other markers in patients or controls. Slight differences were observed in the ABO distribution between nulliparous and parous women, between young versus old ages at first birth, and between pre- and postmenopausal women, but these differences were the consequence of the age effect. No differences were noted among patients, sisters, and controls with regard to chromosome aberrations, C- and G-band variants, or sister chromatid exchanges.

Significance for Cancer Research (NCP Objective 5 Approach 5.1)

Project Officer: Joseph Fraumeni, M.D., Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 1/28/76
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Relationship of Thyroid Function to Growth of Mammary Tumors

Principal Investigator: David Goodman, Ph.D.
Name/Address Albany Medical College
Performing Organization: Albany, New York 12208

Contract Number: NO1-CB-74107
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To determine whether hypothyroidism is a significant risk factor for breast cancer.

Approach: Hypothyroidism will be induced by iodine deprivation or propylthiouracil administration in Sprague-Dawley rats. Determine the effect of hypothyroidism on initiation and growth of mammary tumors induced by DMBA administered intragastrically or intravenously prior to and following induction of the hypothyroid state. Effects of hypothyroidism on inquinal mammary glands exposed directly to DMBA are planned. The possible role of increased concentration of plasma TSH will be studied. Estrogen dependence and prolactin binding of the DMBA-induced tumors in hypothyroid and euthyroid animals will be compared.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak Site Visit Date: 4/12/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $34,200

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acids on the Biological Properties of Mammary Cancer

Principal Investigators: Dr. Janet S. Butel, Dr. Florence Farber
Name/Address Baylor College of Medicine
Performing Organization: Houston, TX

Contract Number: NO1-CB-53904
Starting Date: 4/1/75 Expiration Date: 3/31/78

Goal: To determine the relationship between transfer of nucleic acid preparations into cells and possible changes in their growth potential.

Approach: The approach to date has been three-fold: (1) To establish well-characterized mouse mammary tumor cell lines in culture, (2) to determine their in vitro growth parameters, and (3) to characterize and optimize conditions for uptake of exogenous DNA by mammalian cells. These studies are preparatory to determining the effect of exogenous DNA on the growth properties of mammary tumor cells.

Progress: Cell lines have been established in tissue culture from mammary tumors which arose in BALB/c or C3H mice either spontaneously or after treatment with a hormone or a chemical carcinogen. Several clonal lines have been derived from each parental line. The following growth parameters were monitored with each cell line: plating efficiency, saturation density, doubling time, and colony formation on plastic or in methylcellulose. The tumor cell lines did not differ markedly in saturation density levels from normal cells. The majority of the cell lines were transplantable in syngeneic weanling mice. There was no single growth property in vitro which correlated with transplantability in vivo. Membrane alterations associated with transformation in other systems are being investigated with the mammary tumor cell lines. The fate of exogenous DNA taken up by representative mammary tumor cell lines has been studied. In contrast to many other cell lines, the mammary tumor cells exhibit an apparent defect in the efficient transport of incorporated exogenous DNA into the nuclear fraction.

Significance for Cancer Research (NCP 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 8/6/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Neoplasia of the Mammary Gland

Principal Investigator: Dr. Daniel Medina
Name/Address Baylor College of Medicine
Performing Organization: Houston, TX

Contract Number: NO1-CB-43907
Starting Date: 6/10/74 Expiration Date: 6/9/78

Goal: Determine the relationship between stromal and other mesenchymal cells on growth and differentiation of normal and malignant epithelial cells from the mouse mammary gland.

Approach: Develop and utilize mammary cell culture systems in which normal and malignant epithelial cells can be co-cultured in presence of mesenchymal cells. Characterize cloned cell strains from mouse HAN and mammary tumor, as well as rat mammary tumor, with regard to growth rates, hormone receptors, precursor incorporation into DNA and protein, and differentiated functions. Study the effect of stromal cells from various sources on the growth of the characterized cells listed above in co-culture or in filter culture. Study the specificity of various mammary fat depots in regulating growth of normal and preneoplastic epithelial cells.

Progress: Multiple cloned strains of preneoplastic and neoplastic rodent breast epithelioid cells have been developed. These include 4 from R3230AC rat tumor, 6 from new DMBA induced tumors in Sprague-Dawley rats, 9 from GR mice and 8 from BALB/c mice. Several strains contain estrogen receptor activity and two exhibit a growth response to estradiol. Ultrastructural studies of many of these cells have demonstrated the presence of desmosomes and microfilaments. Most epithelioid cell strains form tumors on reinjection into animals. Fibroblast-like cells appear to inhibit estradiol binding when grown on the opposite side of Nucleopore filters in a special Mark II Rose chamber. Epithelioid GR mouse tumor cells appear to exert a toxic effect on GR mouse fibroblasts grown on the opposite side of the filter as demonstrated by ^{3}H-thymidine incorporation and the scanning electron microscope. The interactions of mouse mammary nodule cells and normal mammary cells on the neoplastic transformation has been examined *in vivo*. Nodule lines were established by serial transplantation of nodule tissue in the mammary fat pad. Dissociation into "single" cells led to enhanced tumor potential, even in the low oncogenic line D1. The interaction of normal mammary cells on the tumor potential was examined in nodule line D2. Mice were injected in the mammary fat pad with either D2 cells or D2 cells plus normal mammary cells (pregnant). Normal cells inhibited markedly the tumor potential of nodule cells. The experiments suggest that normal mammary cells can inhibit the neoplastic transformation in nodule cells.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Richard A. Knazek, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/15/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $87,500

CONTRACT RESEARCH SUMMARY

Title: Effects of Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator: Jeffrey M. Rosen, Ph.D.
Name/Address Baylor College of Medicine
Performing Organization: Houston, Texas 77030

Contract Number: NO1-CB-74189
Starting Date: 8/29/77 Expiration Date: 8/28/78

Goal: To study the influence of perinatal estrogen and progesterone administration on the development and expression of differentiated function in the murine mammary gland.

Approach: Newborn BALB/c mice will be injected with estrogen or progesterone or with both hormones. The organ cultured mammary glands will be exposed to: (a) insulin, hydrocortisone, and prolactin; (b) various doses of progesterone; (c) carcinogen and non-carcinogen analogues of DMBA; (d) thyroxine and growth hormone. Perinatal hormone treatment in virus-positive and-negative BALB/c strains of mice will be compared. Morphological and biochemical analysis including determinations of casein mRNA, and casein will be done.

Progress: New Contract

Significance for Cancer Research (NCP Objective 1 Approach 1)

Project Officer: Chester V. Piczak Site Visit Date: 3/1/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $70,100

CONTRACT RESEARCH SUMMARY

Title: Role of Prolactin in Breast Cancer

Principal Investigator: Dr. Olof H. Pearson
Name/Address
Performing Organization: Case Western Reserve University
Cleveland, OH

Contract Number: NO1-CB-23859
Starting Date: 6/26/72 Expiration Date: 6/25/77

Goal: To find specific receptor sites for prolactin, estrogens and progesterone which might predict hormone responsiveness of individual breast tumors.

Approach: Human and animal mammary tumor tissue for the following studies: (1) Detect and quantify prolactin receptors in membrane fractions of mammary tumors in animals and humans; (2) measure estrogen and progesterone receptors in mammary tumors; (3) correlate receptor findings in tumors with response to treatment with antiprolactin drug (Lergotrile), with antiestrogen drug (Tamoxifen) and ablation (hypophysectomy).

Progress: Prolactin (PRL) receptors are abundantly present in DMBA-induced rat mammary cancers and are present in low titers in about 30% of human mammary cancers. A significant correlation exists between the presence of PRL receptors and hormone dependence in DMBA tumors. Hormonal factors influence the number of PRL binding sites in rat mammary cancers and in liver, and PRL appears to play a major role in regulating its own receptor sites. When estrogen receptors (ER) in DMBA tumors are blocked by antiestrogen (Tamoxifen), tumor growth is readily stimulated by endogenous PRL indicating a primary role of PRL in maintaining the growth of this tumor. ER measurements in human mammary cancers are predictive of the response of the patient to hypophysectomy and to antiestrogen (Tamoxifen) treatment. Progesterone receptor (PgR) measurements in human mammary tumors have not added significantly to the prediction of response. Antiestrogen treatment (Tamoxifen) induces remission in patients with breast cancer and the results are approaching those of hypophysectomy. Tamoxifen has induced significant tumor regression in post-hypophysectomy patients who have undetectable levels of PRL suggesting that estrogen has a direct growth stimulating role in human breast cancer. Anti-PRL-treatment (Lergotrile) has failed to induce objective tumor regression in 12 patients with breast cancer despite good suppression of serum PRL. However, Lergotrile fails to suppress growth hormone secretion and HGH is known to have lactogenic properties. Hypophysectomy after Tamoxifen treatment is being carried out to determine whether further tumor regression can be obtained by removal of pituitary hormones.

Significance of Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/18/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Pathological History of the Mammary Gland in Pseudohermaphroditic Rats and Mice

Principal Investigator: Drs. Susumu Ohno and James Kan
Name/Address City of Hope National Medical Center
Performing Organization: Duarte, CA

Contract Number: NO1-CB-33907
Starting Date: 6/27/73 Expiration Date: 6/26/78

Goal: To study the underlying mechanism of the inhibitory action of testosterone on mammary gland development by the use of the X-linked Tfm (testicular feminization) mutation of the mouse.

Approach: In mice and rats, development of mammary carcinoma as well as normal lactation by nursing mothers depend upon the presence of the unimprinted feminine type hypothalamo-pituitary-ovarian axis. The current view that the neonatal masculinization of the central nervous system is done, not by testosterone itself, but by estradiol derived in situ from testosterone, can be tested on Tfm/Y mice, for their mutated brain lacks functional androgen-receptor, while being endowed with the normal estrogen-receptor. More recently, it was found that female mice, immunized against the Y-linked histocompatibility (H-Y) antigen, fail to lactate while maintaining normal fertility.

Progress: A single intragastric dose of 20 mg of DMBA (7,12-dimethylbenz-(a)anthracene), administered to normal female rats, induced mammary carcinomas in virtually 100% of them. Female rats with the neonatally masculinized central nervous system show an almost complete resistance to the DMBA induction of mammary carcinomas. The neonatal brain of mutant Tfm/Y mice lacks the functional androgen-receptor, while being endowed with the normal estrogen-receptor. With the Sloan-Kettering group, evidence was accumulated which supports the proposal that H-Y antigen is the long sought-after product of the mammalian Y-linked testis-determining gene.

Significance for Cancer Research (NCP Objective 1 Approach 4)

Project Officer: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/10/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $92,700

CONTRACT RESEARCH SUMMARY

Title: Protein Nucleic Acid Interactions in Transcription of Normal and Malignant Cells

Principal Investigator: Dr. Kenneth S. McCarty
Name/Address Duke University
Performing Organization: Durham, NC

Contract Number: NO1-CB-23857
Starting Date: 6/29/72 Expiration Date: 12/28/76

Goal: To elucidate the role of proteins of the mammary cell nucleus in the expression and regulation of genetic information of normal and malignant cells.

Approach: To improve techniques for isolation of chromatin and extraction of acidic nuclear proteins. DNA-protein rebinding techniques to be used with isolated nuclear proteins to determine the comparative binding affinities of the phosphorylated vs dephosphorylated acidic chromosomal proteins. Phosphorylation and acetylation of acidic chromosomal proteins were compared in preparations from template active and inactive fractions utilizing techniques of sucrose density gradients, solubility in DNAase II-2 mM Mg^{++}, and ECTHAM cellulose columns. These preparations were also used to compare binding of activated cytoplasmic steroid receptor complex interactions with mammary chromatin from (1) normal glands of Fisher 344 rats and Heston C_3H mice and (2) adenocarcinoma of rat 13762, R3230 AC and mouse BA or H2712 tumors.

Progress: Developed techniques for preparation of nuclei and chromosomal protein fractions from Fisher 344 rat mammary glands. Optimum purity and yield of nuclei for in vitro transcription required several factors; 0.5 hexylene glycol and NP 40 were required to stabilize nuclei and to strip rough endoplasmic reticulum and nuclear membranes. Protease inhibitors are essential in the studies. Nucleosomes from these preparations were compared. DNA synthesis in mammary gland explants from hormone-primed virgin females was found to be superstimulated in vitro by 10^{-8}M estradiol and 10^{-6}M progesterone when cultured in medium containing insulin, dexamethasone and placental lactogen (I,D,PL; 5 µg/ml each). These steroids likewise increased ^{32}P-casein formation in vitro in I,D,PL medium. Histone H2a was fractionated electrophoretically into multiple components exhibiting differing degrees of hormonally induced phosphorylation. An apparatus was developed for mass culture of surface contacted mammary epithelial cells to provide synchronized cell populations of hormone responsive cells. Several papers were published in this contract period by McCarty and colleagues.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 7/25/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Prevent the Formation and Progression of Mammary Gland Preneoplastic Lesions

Principal Investigator: Richard C. Moon, Ph.D.
Name/Address IIT Research Institute
Performing Organization: Chicago, Illinois 60616

Contract Number: NO1-CB-74207
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To study the use of retinoids to prevent the development and/or progression of preneoplastic lesions of the mammary gland.

Approach: Non toxic doses of synthetic analogues of vitamin A (retinoid) will be administered to experimental animals in beadlet form in the diet to study: the effect on incidence of HAN and spontaneous mammary tumors in C_3H/He mice and DMBA-induced hyperplasias and tumors in BALB/c mice. The prevention of the progression of these ductal lesions and DMBA-induced mammary tumors are planned. Similar studies are planned using the DMBA-induced lesions in the Sprague-Dawley rat. Examine the effect of retinoids on carcinogen uptake and binding and effect of time of initiation of retinoid treatment on DMBA-induced carcinogenesis in the Sprague-Dawley rat. Studies will be designed to investigate the mechanisms by which retinoids are effective.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak Site Visit Date: 4/21/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $81,900

CONTRACT RESEARCH SUMMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Dr. Klaus Kratochwil
Name/Address Institute for Molecular Biology
Performing Organization: Salzburg, Austria

Contract Number: NO1-CB-33883
Starting Date: 6/29/73 Expiration Date: 3/28/78

Goal: To study the acquisition of hormone responsiveness during development of the mammary gland. Analysis of the androgen response with special emphasis on the role of tissue interaction.

Approach: Determine androgen-sensitive phase of mammary rudiment by exposing explanted mammary buds of various developmental stages to testosterone in vitro. Determine the target tissue for the hormone by experimental tissue combinations (gland epithelium and surrounding mesenchyme) taking advantage of the androgen-insensitive Tfm-mutant. Find out how the primary target tissue affects its partner during the androgen response (methods: experimental tissue combinations, electron microscopic and histochemical study of the response itself, autoradiography of suitable precursors, especially of tissue interface material). Determine whether the duration of the androgen-sensitive phase of the gland is specified by transient hormone responsiveness of the (primary) target tissue or by changing properties of its partner (hormone binding in relation to responsive phase, combination of tissues in different developmental stages).

Progress: Established that the response to testosterone of explanted mammary gland rudiments is identical to that found in male fetuses. The androgen-sensitive phase in mammary gland development was found to last only from day 13 to day 15 of gestation, acquisition and loss of responsiveness occurring equally in explanted rudiments. Further established that the target tissue for testosterone is the mesenchyme and not the epithelium. From this follows that the necrosis of the gland epithelium, as seen in the electron microscopic study, is not a direct consequence of hormone action but must be caused by testosterone-activated mesenchymal cells. The mechanism by which mesenchymal cells attack the gland epithelium still remains enigmatic, although it was found during the last year that this attack is strictly tissue specific and does involve the breakdown of tissue interface material (basal lamina).

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 9/20/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $60,000

CONTRACT RESEARCH SUMMARY

Title: Influence of Mammary Carcinoma Cells on the Formation and Absorption of Collagen

Principal Investigator: Gerhard Wirl, Ph.D.
Name/Address Institut for Molekularbiologie
Performing Organization: A-5020 Salzburg, Austria

Contract Number: NO1-CB-74109
Starting Date: 9/1/77 Expiration Date: 8/31/78

Goal: To isolate and characterize collagenase and inactive forms of collagenase present in mammary tumor tissue.

Approach: Studies are designed to: (a) extract collagenase directly from DMBA-induced tumors in the rat and spontaneous tumors in C_3H mice as well as from tumor tissue cultured media; (b) purify the enzyme and examine its purity using disc gel electrophoresis and immunodiffusion; (c) detect the activity of the enzyme; (d) determine the distribution of the enzyme in the tumor; (e) find any inactive form of the enzyme as a proenzyme; (f) determine the production of collagenase by epithelial cells or stromal cells. Studies of human tissues depends on results obtained from the mouse and rat.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak Site Visit Date: None
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $41,600

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Cultures of Normal Mammary Epithelial Cells

Principal Investigator: Dayton S. Misfeldt, Ph.D.
Name/Address Leland Stanford Jr. University
Performing Organization: Stanford, California 94305

Contract Number: NO1-CB-74094
Starting Date: 7/1/77 Expiration Date: 6/30/78

Goal: To isolate and maintain growth of pure normal mammary epithelial cells in culture.

Approach: Remove mammary glands from virgin BALB/c mice hormonally stimulated for an optimal period and utilize an optimal method for gland dissociation to determine the seeding efficiency and growth confluency using: (a) single cells and clumps of cells of known and varying size; (b) various commercially available nutrient media; (c) various types of sera over a range concentration of 1-20%; (d) different pH levels, O_2 tension; (e) optimal substrata as treated and untreated glass bottles and plastic culture dishes.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 4)

Project Officer: Chester V. Piczak Site Visit Date: None
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $63,500

CONTRACT RESEARCH SUMMARY

Title: Animal Mammary Tumors and Human Cell Bank Facility

Principal Investigator: Dr. Arthur E. Bogden
Name/Address Mason Research Institute
Performing Organization: Worcester, MA

Contract Number: NO1-CB-74175
Starting Date: 6/28/63 Expiration Date: 6/27/78

Goal: To maintain as a service facility a bank of biologically characterized and monitored (a) in vivo transplantable animal mammary and endocrine-related tumors, and (b) human and animal breast tumor cell lines established in in vitro culture, to be used by the BCTF and other selected investigators.

Approach: Freeze-preservation of mammary and endocrine-related tumors of animal origin, as well as human breast tumor cell lines: (a) submittal of tumors and cell lines by qualified investigators able to furnish pertinent background information; (b) biological characterization of transplantable tissues, both pre- and post-freeze preservation, by determination of growth curves, specific effects on host organs, host survival, serum hormone levels, histology, karyology, response to ablative procedures; (c) cell lines tested for viability, plating efficiency, freedom from contamination and tumorigenicity by nude mouse implantation; and (d) characterized tissue and cell lines preserved in Linde liquid nitrogen freezers according to well-recognized, proven procedures.

Progress: During the past contract period 122 tumors have been shipped to 62 investigators; 48% were of mammary and 14% of anterior pituitary origin. There are 118 different tumors or sublines stored in 2,467 ampules in liquid nitrogen. Five new tumors and 27 tumor lines have been maintained in serial transplantation for characterization and/or replenishment of Tumor Bank inventory. Four laboratories have sent personnel to observe transplantation and preservation techniques. Human mammary cell lines now characterized and freeze-preserved free of mycoplasma are the MDA-MB-134, 157, 175, 231, 330, 361, 415, HBL-100, MCF-7, BT-20, SW-613. Thirty-three shipments, which include viable cultures, frozen ampules and/or gram quantities of packed cells, have been made to 21 different investigators. For cell line individualization HL-A antigen and pleomorphic isoenzyme profiles are being defined. A breeding colony of the nude athymic mice has been established in a pathogen-free facility for testing the tumorigenicity of the cell lines.

Significance for Cancer Research (NCP Objective 3,4,5 Approach_____)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/4/71
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $169,800

CONTRACT RESEARCH SUMMARY

Title: Identification of Mammary Tissues

Principal Investigator: Dr. Melvyn S. Soloff
Name/Address Medical College of Ohio
Performing Organization: Toledo, OH

Contract Number: NO1-CB-63983
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To isolate, identify, characterize, and quantitate myoepithelial cells in rat mammary tissue and tumors of rat and human origin.

Approach: Separate myoepithelial cells by centrifugation in albumin gradients, using oxytocin binding, and scanning/transmission electron microscopy to identify the cells. Examine the potential of using alkaline phosphatase and ATPase as cell markers. Prepare specific antiserum and identify, quantitate, and characterize myoepithelial cells in mammary glands from virgin, pregnant, and postpartum rats, and in rat mammary tumors induced with DMBA. Parallel investigations will be carried out with human tumors obtained from surgery.

Progress: Immunochemical and histochemical assays accomplished. Specific discrimination of myoepithelial cells from other mammary cell types achieved in mammary slices and partially purified cell preparation. Discrimination of cell types also indicated by EM and SEM. Indication from ^{3}H-oxytocin studies of human mammary tissue/tumors that human myoepithelial cells can be estimated, particularly with fibrocystic disease (sclerosing adenosis). Oxytocin binding of mammary gland followed through pregnancy and lactation was found to progressively increase except for transient decline at parturition. Treatment of cells with oxytocin produces observed contraction of myoepithelial cells.

Purification of myoepithelial cell populations beyond current level of 60% has been limiting. Major contaminant appears to be heavy species of secretory cell. The latter form mixed cell aggregates with myoepithelial cells. Immunoassay for localization of oxytocin binding or quantitation of same indicates some weak activity in non-myoepithelial cells.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak, B.S., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/13/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $98,000

CONTRACT RESEARCH SUMMARY

Title: Prevent the Formation and Progression of Mammary Gland Preneoplastic Lesions

Principal Investigator: Clifford W. Welsch, Ph.D.
Name/Address Michigan State University
Performing Organization: East Lansing, Michigan 48824

Contract Number: NO1-CB-74090
Starting Date: 8/29/77 Expiration Date: 8/28/78

Goal: To prevent the early development of preneoplastic mammary gland lesions and progression of these preneoplastic lesions to neoplastic lesions by chronic suppression of pituitary prolactin secretion.

Approach: A prolactin-suppressing drug, 2-bromo-α-ergocryptine (CB-154) will be administered prior to and after the spontaneous development of, or the induction of mammary gland dysplasias. The following mammary gland dysplasias will be examined: A. Mice: 1) spontaneously occuring alveolar lesions (HAN) -DBA/2; 2) pregnancy-induced alveolar lesions (HAN) - A/J; 3) DMBA-induced lesions (DH) - BALB/c; 4) pregnancy-incuded ductal lesions (plaques) - GR/A. B. Rats: 1) DMBA-induced ductal lesions - Lewis and Sprague-Dawley; 2) X-irradiation-induced ductal lesions - Lewis and Sprague-Dawley; 3) DMBA-induced alveolar lesions - Lewis and Sprague-Dawley; 4) X-irradiation-induced alveolar lesions - Lewis and Sprague-Dawley; 5) pregnancy-induced alveolar lesions - Sprague-Dawley.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak Site Visit Date: 4/21/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $89,450

CONTRACT RESEARCH SUMMARY

Title: Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces

Principal Investigator: Dr. Kermit L. Carraway
Name/Address Oklahoma State University
Performing Organization: Stillwater, OK

Contract Number: NO1-CB-33910
Starting Date: 6/27/73 Expiration Date: 10/31/76

Goal: To study composition and biochemical properties of normal and neoplastic mammary cell plasma membranes.

Approach: Single cell suspensions from normal lactating mammary gland and tumors of rats will be prepared by enzymatic procedures and fractionated on various gradients or prolactin affinity columns. Cell surface repair will be evaluated by incorporation of radioactive fucose, glucosamine, and leucine. Surface structures of normal and tumor membranes will be evaluated by gel electrophoresis, glycoprotein patterns, lectin perturbation, susceptibility to proteolysis, and iodination with lactoperoxidase. The purity of isolated cell membranes will be assessed by biochemical and microscopic methods. Quantitative binding of prolactin to cell membranes will be determined.

Progress: Purified plasma membrane fractions were prepared from lactating mammary tissue and R3230AC adenocarcinoma by fractionation of a tissue microsomal preparation, followed by subfractionation after digitonin treatment. Plasma membranes were prepared from 13762A ascites adenocarcinoma after zinc stabilization. Cell surface 5'-nucleotidase and ATPase enzymes of the ascites cells were characterized and their perturbation by concanavalin A investigated. Cooperativity in the inactivation by Con A appears to relate to the association of the enzyme with the membrane and its modulation by cellular cytoskeletal elements. The apparent ATPase activation by Con A is actually a relief of substrate inhibition and may serve as a control mechanism for regulating external ATP concentration. The major glycoprotein of the ascites cells has been isolated and is being characterized. Prolactin receptor for rabbit mammary particles was solubilized and progress has been made in its purification. Dissociation of prolactin from its receptor in particles has been examined.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/8/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of the Mammary Cell Surface

Principal Investigator: Dr. Eugene A. Davidson
Name/Address
Performing Organization: Pennsylvania State University
Milton S. Hershey Medical Center
Hershey, PA

Contract Number: NO1-CB-63984
Starting Date: 6/20/76 Expiration Date: 6/19/78

Goal: To isolate and characterize the cell surface glycoproteins and other complex carbohydrates produced by human mammary carcinoma cells.

Approach: Apply techniques for surface labeling of the protein and carbohydrate moieties of the glycoproteins on the cell plasma membrane. Assess the labeled macromolecules in terms of molecular size and localization within the membrane. Apply the best method for isolation and purification of plasma membranes. Characterize the purified plasma membrane for their chemical architecture, biological, immunological properties, and enzyme markers. Human mammary cell lines obtained from the BCTF Cell Bank will be used for the studies.

Progress: Two tumorigenic lines, MCF-7 and MDA-MG-231, and a human normal line (HBL-100) were established in culture. Growth data were acquired and several alternative media were tested for their growth. The cells were cultured in roller bottles as a prelude to large-scale cultivation for plasma membrane and glycoprotein isolation. Morphological characteristics were established by direct microscopy, and surface properties were examined by scan electron microscopy and transmission electron microscopy using ruthenium red-stained preparations; the production of lactose by all three cell lines was documented.

Preliminary isotopic labeling of these cells was carried out utilizing tritiated glucosamine and ^{35}S-inorganic sulfate as markers. Fractionation of the labeled complex saccharides was achieved and characterization of several of the products was completed both for normal and tumorigenic lines. Some specific differences were noted in these experiments.

Several experiments are currently under way to establish the feasibility of growing these cells in nude athymic mice as solid tumors and as ascites cells.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/17/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $114,500

CONTRACT RESEARCH SUMMARY

Title: Cultivation of Normal and Malignant Human Mammary Tissue

Principal Investigator: Dr. Edwin V. Gaffney
Name/Address Pennsylvania State University
Performing Organization: University Park, PA

Contract Number: NO1-CB-33898
Starting Date: 6/29/73 Expiration Date: 3/27/78

Goal: Establish monolayer cultures of human mammary epithelial cells derived from tissue and breast secretion samples. Examine the influence of hormones including prolactin and estradiol in mammary cell differentiation and/or transformation.

Approach: Isolate cells. Measure cell number, DNA, general protein and phosphoprotein synthesis in cultures of normal and malignant cells. Normal cells grown in hormones and/or carcinogens. Cell characterization by electron microscopy.

Progress: Epithelial cell cultures have been established from normal and malignant tissues and secretions derived from lactating breasts. The biological characteristics and observations on the hormone responsiveness of these cells in culture have been reported in two papers. In general, epithelial mammary cells growing in colonies show a mitogenic response to insulin after 48 hours and a similar but delayed response to prolactin. Prolactin induces several ultrastructural changes and enhances the synthesis of phosphoproteins in normal cells. The two cell types observed in breast milk originated through exfoliation from the cell linings of ducts and alveoli. Some cultured cells appear to be actively engaged in the synthesis of milk constituents while others are inactive and structurally identical to cells derived from reduction mammoplasty specimens. The milk cell system will be used to study the hormone controlled differentiation of inactive to active human cells. Two cell lines were established from primary cultures of human milk. An extensive study of the characteristics of one line (HBL-100) demonstrated that these cells may be classified as (1) epithelial, originating from a normal source; (2) mammary, as inferred from their capacity to synthesize lactose; and (3) transformed, but not malignant.

Studies with malignant cells revealed that fibroblast-free cultures can be established from only certain tumor types. Malignant cells require serum and were observed to grow better in McCoy's 5a or Waymouth's MB 73/1 media when compared to 4 other common media. Preliminary studies on the hormone responsiveness of malignant cells have shown a tendency to grow more rapidly and produce greater amounts of protein following maintenance in prolactin. Current experiments focus on extending these preliminary findings with hormones.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/27/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $68,600

CONTRACT RESEARCH SUMMARY

Title: Isolation of Prolactin Cells from Human and Rat Adenohypophysis

Principal Investigator: Dr. Wesley C. Hymer
Name/Address Pennsylvania State University
Performing Organization: University Park, PA

Contract Number: NO1-CB-23863
Starting Date: 6/28/72 Expiration Date: 3/26/78

Goal: To isolate mammotrophs from human and rat adenohypophysis, to cultivate them and study their effects on mammary tissue, normal and neoplastic.

Approach: The following material will be used: (1) Human pituitaries from surgical material, and (2) rat pituitaries from animals bearing either R-13762 mammary adenocarcinomas (ascites and solid form) or R-3230 tumors. Experiments include (1) cell dissociation, (2) cell separation by velocity sedimentation at 1 g (NIH model chamber), (3) cell cultures, and (4) radioimmunoassay of pituitary hormones.

Progress: I. Effect of tumor on pituitary of host. Intracellular contents of prolactin (PRL), GH and TSH are reduced in animals bearing R-13762 tumors, whereas FSH and LH levels are often elevated. Animals with R-3230 tumors show normal PRL levels. Both small and large PRL cells are present in glands of controls; only small cells are in glands from animals with 13762 tumors, whereas those from R-3230 tumor-bearing animals are similar to controls. Regression of the tumor induced by intragastic administration of phenylalanine mustard (PAM, 2 mg/kg) 3x/week leads to partial restoration of PRL cell function in the 13762, but not the 3230, tumor model. These results suggest that tumor suppression of the pituitary may be partially specific to tumor type. II. Effects of PRL on 13762 mammary ascites tumor (MAT) cells. Daily injections of ovine PRL IP into animals bearing MAT tend to (a) prolong life of some animals (as does E_2, Dr. A. Bodgen), (b) tumor cell size, (c) ^{3}H-thymidine uptake, (d) promote cell clumping, (e) electrophoretic mobility of the cells, and (f) remove the "fuzzy coat" on the surface of microvilli. Reproducible separations of MAT cells into subpopulations of different size have been achieved by elutriation. III. Cell culture. The extent of PRL secretion from pituitary cells of 7 breast cancer patients varied by 200-300% depending upon medium and serum. In 5 exps. addition of E_2 (300 pg/ml) significantly stimulated PRL secretion, thus documenting a positive effect of the steroid on the mammotroph in vitro.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/3/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $85,000

CONTRACT RESEARCH SUMMARY

Title: Studies on Basic Biological Events in the Pathogenesis of Mammary Cancer

Principal Investigator: Dr. Thomas L. Dao
Name/Address: Roswell Park Memorial Institute
Performing Organization: Buffalo, NY

Contract Number: NO1-CB-23865
Starting Date: 6/15/72 Expiration Date: 6/14/77

Goal: To determine the role of hormones and other host factors in the pathogenesis of mammary dysplasia and neoplasia.

Approach: (1) Continue characterization of hyperplastic alveolar nodules (HAN) of rat mammary tissue by cell kinetics consisting of cell proliferation rate in vivo, DNA synthesis, and cell cycle; karyotyping of chromosomes and their banding patterns. (2) Investigate the fate of HAN by transplantation of individual HAN into subscapular fat pad of isologous recipient host and observe whether neoplastic transformation occurs; transplanting the whole mammary gland (with HAN) into the interscapular fat pad and following HAN growth; and studying the incidence of HAN in aging rats and determining their natural history. (3) Determine the malignant potential of HAN in vitro by exposure to low levels of DMBA and transplanting in vivo; also in vivo experiments with whole mammary gland (with HAN) transplanted into isologous host and host treatment of DMBA (low level). (4) Study the effect of DMBA metabolites on the induction of HAN in vivo and in vitro.

Progress: Have successfully cultivated normal, neoplastic and HAN mammary tissue from Sprague-Dawley rats for long periods. Developed a technique for identification of HAN in vivo so they can be removed for biochemical and transplantation studies. Hormone responsiveness of the three kinds of tissue have been compared biochemically using insulin, estradiol, progesterone and prolactin; the data suggest that HAN contain cell populations which are not in proliferation as compared to those of mammary gland or the tumor. DMBA given intravenously to "old" rats develops numerous HAN but no mammary tumors, while the same carcinogen given by local application induces mammary tumors but no HAN. The data do not support the concept that HAN is an intermediate step in tumorigenesis of the mammary gland in the rat. DMBA-induced mammary tumors have been demonstrated to be of ductal origin. HAN transplanted into the interscapular fat pad develop either lobuloalveolar or ductal growth but not neoplasia. Biochemical characterization of HAN shows a lack of hormone dependence. A comparative chromosomal study of normal mammary tissue, HAN and mammary tumors indicates that both normal mammary gland and HAN have normal diploid karyotype patterns. On the other hand, mammary tumors show frequent abnormalities of both chromosome number and structural aberrations.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Studies on the Prevention of Metastasis in Mammary Cancer

Principal Investigator: Dr. Untae Kim
Name/Address Roswell Park Memorial Institute
Performing Organization: Buffalo, NY

Contract Number: NO1-CB-23864
Starting Date: 6/15/72 Expiration Date: 8/31/76

Goal: Determine the mechanism of mammary tumor metastasis in relation to antigen-shedding property of tumor cells and host immune responses, and to develop clinically applicable procedures for prevention of metastases.

Approach: Utilizing established spontaneously metastasizing and nonmetastasizing mammary tumors in W/Fu rats, the following lines of research are being pursued: (1) Biochemical analyses of tumor cells to learn the mechanisms of antigen-shedding with respect to glycoprotein and glycolipid metabolism in isolated plasma membranes, and the role of cyclic nucleotides and lysosomal enzymes. (2) Analysis of host immune responses in relation to in vitro migration pattern of peripheral lymphocytes, spleen cells and thymocytes from tumor hosts, and to study localization of free or bound antibodies in sera and on the surface of tumor cells and lymphocytes. (3) Development of clinically practical, experimental therapeutic modalities for prevention of metastasis. (4) Establishment of biochemical guidelines for simple vs. radical mastectomy.

Progress: (1) Pathogenesis of spontaneously metastasizing mammary tumors and their induction method have been established. (2) Surface antigen-shedding property has been shown to be responsible for loss of immunogenicity and acquisition of metastasizing capacity. (3) Inverse relationship between metastasizing capacity and levels of plasma membrane marker enzymes has been established. (4) Direct correlation between endogenous cAMP and metastasizing capacity, and inverse relationship with cAMP phosphodiesterase have been found. (5) Metastasizing tumor cells have greatly increased glycoprotein turnover as evidenced by elevated glycosyltransferase levels in tumor cells, plasma membranes, and host sera. (6) Qualitative difference in cellular immune response between lymphocytes from metastasizing and nonmetastasizing tumor host as well as quantitative difference in humoral immune response have been found.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Change in Mammary Sensitivity to Carcinogens Related to Host Physiological State

Principal Investigator: Dilip K. Sinha, Ph.D.
Name/Address Roswell Park Memorial Institute
Performing Organization: Buffalo, New York 14263

Contract Number: NO1-CB-74105
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To investigate the susceptibility of the mammary gland to neoplastic transformation during changing conditions of the host due to age and pregnancy.

Approach: Study the DNA synthesis of ductal and alveolar cells of prepubertal, pubertal and postpubertal rats. Attempt to induce tumors in old mammary glands transplanted into gland-free mammary fat pads of old rats. Compare induction of tumors in serially transplanted mammary glands with tumorigenesis in 50 day old rats. Determine the physiological state of the mammary gland undergoing cell division before milk protein synthesis. Study tumorigenesis of mammary glands in different physiological states using in vitro and in vivo systems. Effect of DMBA on milk protein synthesis will be determined.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 2,3)

Project Officer: Chester V. Piczak Site Visit Date: 4/22/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $63,200

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: Drs. W.B. Dandliker and S.A. Levison
Name/Address Scripps Clinic and Research Foundation
Performing Organization: La Jolla, CA

Contract Number: NO1-CB-43905
Starting Date: 6/1/74 Expiration Date: 2/28/78

Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones. Investigate possible competitive, cooperative or sequential effects in the binding of hormones to their specific cellular receptors of mammary tissue. Based upon these findings, develop simple fluorescence receptor assays useful in the clinical laboratory.

Approach: Fluorescent tagging of hormones, e.g., prolactin, growth hormone, insulin, estradiol, testosterone, corticosteroids, and purification of the fluorescent derivatives by chromatography. Specificity of the fluorescent tagged hormone tested by its ability, as compared to the unlabeled hormone, to displace the radiolabeled hormone in charcoal and sucrose density methods. Equilibria and kinetics of hormone binding to specific receptors monitored by changes in fluorescence polarization or by visualization of the labeled hormone in the fluorescence microscope. Utilize microsomal and cytosol receptors as well as whole cells from mammary tissues and mammary tumor lines.

Progress: Fluorescence polarization studies of the specific binding of prolactin and estradiol to isolated receptors and to whole cells are being pursued by equilibrium, kinetic and dilution jump techniques. Scatchard plots indicate the presence of a wide range of binding affinities in receptor sites or the presence of strong cooperative effects. The complexity of these systems is such that equilibrium measurements alone yield only limited information. Further knowledge in this area can be obtained both by receptor purification and by kinetic techniques including especially dilution jump. As an important adjunct to these studies, the effects of fluorescence labeling on the biological specificity of hormones are being investigated. Estradiol was labeled in the 6 position with fluorescein utilizing two different chemical spacers (between the estradiol and fluorescence molecules), and one derivative has been made in the 17 position. The labeled hormones as well as various intermediates in the synthesis are being tested as competitors with radiolabeled estradiol for cytosol receptors sites as measured by the charcoal and sucrose gradient methods. As an example, estradiol, fluorescein labeled in the 17 position, is 80% as effective as native estradiol in the charcoal test. Derivatives are being sought which show specificity not only in isolated receptor assays but also in the intact cell.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/13/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $92,000

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acids on the Biological Properties of Mammary Cancer

Principal Investigator: Dr. Aaron Bendich
Name/Address Sloan-Kettering Institute for Cancer Research
Performing Organization: New York, NY

Contract Number: NO1-CB-43904
Starting Date: 6/28/74 Expiration Date: 3/27/78

Goal: To ascertain whether in vitro growth characteristics of normal and tumor cells can be modified by nucleic acid preparations and whether these properties are fruitful monitors for in vivo disease.

Approach: To study in vitro (a) the effect of nucleic acids or sperm on the biological properties of normal and tumor cells of animal and human origin, and on explant outgrowths of freshly obtained tissue; (b) the possible induction or alteration of embryonal, fetal or placental gene expression after nucleic acid- or sperm-cell interaction; (c) immunochemical determination of embryonal, fetal, placental or ectopic gene expression in animal or human tumor explants taken from animal hosts or directly from human mammary tissue; (d) whether the sperm-induced alterations in target somatic cells are due to the DNA or to the extrachromosomal DNA-generating components contained within the sperm nucleus; and (e) the refinement or development of methods to achieve (a) to (d).

Progress: When exposed in vitro to human or mouse sperm, penetration of established mammary tumor cells, SH-2, occurred. On subsequent replication, the cells showed increased plating efficiency in liquid and semi-solid media, and an increase in polynucleation which was further enhanced by cytochalasin B, all of which are regarded as measures of oncogenic potential. These effects were decreased when the SH-2 cells were treated instead with human spleen or calf thymus DNA. Hamster cells acquire the ability to express mouse or rat fetal antigens after interaction with mouse or rat sperm, respectively, but SH-2 progeny did not express mouse fetal antigens after incubation with mouse sperm. Several human tumor cell lines and primary tissue explants were tested with antisera made against various human fetal and placental antigens, and a few gave positive immunofluorescence reactions. Human and animal sperm heads were found to contain a DNA polymerase-endogenous RNA complex. Studies were begun to determine whether the sperm-induced alterations of tumor cells, and the oncogenic-like changes in normal cells, are due to the complex, the sperm chromatin, or some other factor(s).

Primary explants of tumor and non-tumor human breast tissues revealed no significant differences in ^{3}H-thymidine uptake. The in vitro labeling frequency of the breast tissue explants was considerably lower than explants of tumors arising in animals inoculated with established tumor cells.

Significance for Cancer Research (NCP Objective 2,2 Approach 3,3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/22/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $87,100 (Terminal)

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Growth of Neoplasms of the Mammary Gland

Principal Investigator: Dr. Merton R. Bernfield
Name/Address Stanford University
Performing Organization: Stanford, CA

Contract Number: NO1-CB-53903
Starting Date: 4/15/75 Expiration Date: 4/14/78

Goal: To define the role of the basal lamina, its constituent glycosaminoglycan (GAG), and specific stromal tissue in mammary morphogenesis occurring postnatally and during pregnancy.

Approach: Mouse mammary glands undergoing morphogenesis will be used to (1) establish the presence of GAG within the epithelial basal lamina, (2) elucidate the pattern of basal laminar GAG distribution and turnover and relate this pattern to epithelial morphogenesis, (3) develop procedures for isolation of epithelia free of stroma but retaining the basal lamina, and (4) establish whether epithelial morphogenesis is dependent upon the basal lamina and stromal tissue.

Progress: The basal lamina of the mammary epithelium contains glycosaminoglycan (GAG). The amount of total and of newly synthesized laminar GAG varies with mammary epithelial development and function. In prepubertal and adult virgin glands, total GAG is distributed uniformly over the epithelial surface; newly synthesized GAG accumulates slowly and has a similar distribution. Total laminar GAG decreases after 10 days and is further decreased after 15 days of pregnancy, especially at sites of lobulo-alveolar growth, and is least during lactation. In contrast, the rate of accumulation of newly synthesized GAG increases during pregnancy and lactation, and the sites of most rapid accumulation are the sites of least amount of total GAG. It appears that laminar GAG exists in a dynamic steady state, that the "turnover" of laminar GAG increases during pregnancy, and varies with the functional state of the epithelium. The ultrastructure of the lamina following ruthenium red staining (for GAG) reveals laminar components in macroperiodic arrays apparently attached to the basal GAG "turnover." Incorporation studies at mid-pregnancy reveal that epithelial and adipose cells differ in the amount and type of GAG produced. Epithelial cells produce 10-fold more total GAG and this GAG is predominantly hyaluronic acid. Adipose cells produce predominantly chondroitin sulfates, small amounts of heparin sulfate and traces of hyaluronate. Postnatal mammary epithelia appear to be similar to embryonic epithelia undergoing morphogenesis in the presence of basal laminar GAG, its turnover, and ultrastructure, as well as in the types of GAG produced.

Significance for Cancer Research (NCP Objective 4, Approach 3)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 9/5/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Biochemical Means by Which Effector Molecules Bind to Mammary Cell Surfaces

Principal Investigator: Dr. Zoltan Lucas
Name/Address Stanford University
Performing Organization: Stanford, CA

Contract Number: NO1-CB-33905
Starting Date: 5/24/73 Expiration Date: 5/23/78

Goal: To study natural mechanisms which impair the host's cellular immune response to syngeneic mammary tumors.

Approach: Determine the immune response to mammary tumors in syngeneic rats with progressive tumor growth. Determine if tumor growth is associated with factors blocking cellular and humoral immunity, investigating soluble [tumor antigen, antibodies to tumor antigen, and anti-(antibody to-tumor antigen)] and cellular (suppressor T- and B-cell) factors.

Progress: In vitro tests show that Fischer rats rapidly develop cellular immunity to the transplanted 13762A adenocarcinoma. Yet, tumor growth is rapid. This may be due to the near simultaneous occurrence of serum blocking factors (BF) which inhibit lymphocyte cytotoxicity in vitro and enhance tumor growth in vivo. By incorporating ^{3}H-fucose into tumor surface antigen(s), a radioimmune assay was developed for both tumor antigen (TA) and antibody to tumor antigen (ATA) based on immune complex formation. Soluble TA, not found in breast cells from lactating rats, induces both cellular cytotoxicity and antibody to the tumor, but contrasts with ascites tumor-cell inoculations by not eliciting soluble BF (7 days after injection). Partially purified TA blocks in vitro CMC when continuously present in the assay, but contrasts with BF from sera of tumor-inoculated animals by having no blocking activity when preincubated with immune cytolytic lymphocytes. BF can be removed by adsorption to the immune lymphocytes but not to nonimmune lymphocytes or tumor cells. These assays permit quantitation of TA, ATA and BF in sera after i.p. injection of 10^{7} tumor cells. BF appears by day 3, rapidly peaking on day 7, then decreasing to about 1/3 of its peak value on day 10. Seventy percent of the blocking in whole sera appears to be due to an immunoglobulin (Ig) whose specificity is not yet established; 30% to free TA. TA and ATA, first detected on day 3, continue to rise exponentially to day 10. The decrease in serum blocking represents a loss in the Ig factor, not free TA. Current formulation of the tumor-host relationship is that this tumor profusely sheds an apparent tumor antigen. High concentration of soluble TA may be sufficient to inactivate immune lymphocytes in the local area of the tumor, and by eliciting anti-receptor antibodies as well as anti-tumor antibodies, specifically abort or modulate a normally sustained immune response, resulting in "tolerance" to the tumor.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/12/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $115,000 (Terminal)

CONTRACT RESEARCH SUMMARY

Title: Control of DNA Synthesis in the Mammary Gland

Principal Investigator: Dr. Frank E. Stockdale
Name/Address Stanford University
Performing Organization: Stanford, CA

Contract Number: NO1-CB-23875
Starting Date: 6/1/72 Expiration Date: 5/31/78

Goal: To determine the role of serum factors and hormones in the initiation and control of DNA synthesis in mammary gland epithelium and mammary neoplasms of mice.

Approach: Studies include (1) the isolation, characterization and source of factors in serum responsible for mammary epithelial growth; and (2) comparison of the proliferative response of normal and malignant mouse mammary gland epithelium in vitro or in vivo to mammary serum factor (MSF) and hormones.

Progress: A factor(s) in serum was found which initiates DNA synthesis in normal mouse mammary gland and in mouse mammary tumor epithelium. This factor--mammary serum factor (MSF)--is relatively heat stable and has been partially purified by ionic exchange chromatography, gel filtration, and isoelectric focusing. Partially purified MSF has a mol. wt. of approx. 11,000 and an isoelectric point of 5.5 to 6.0. MSF can be isolated from a variety of sera, but it is most active on mouse mammary epithelium when isolated from mouse or rat sera. Its activity decreases at least 50% in the sera of hypophysectomized rats and the sera activity is not restored by treating the hypophysectomized rats with estrogen, prolactin, or growth hormone. No detectable change in MSF serum activity occurs with pregnancy or lactation. Very high concentrations of serum are required to initiate DNA synthesis in mammary epithelium from late pregnant and lactating mice and in mammary tumors, but all are equally responsive to insulin.

Present work: (1) Further characterization of mammary serum factor. (2) Studies on the effects of injection of partially purified MSF on mammary gland and tumor growth. (3) Studies on the hypothesis that, in terms of growth, the mammary gland is composed of two or more operationally different populations. (4) Studies on growth-promoting substances released from normal and malignant mammary epithelium.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/10/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $90,000

CONTRACT RESEARCH SUMMARY

Title: Study Breast Cancer Transplantability in Nude-Athymic Mice

Principal Investigator: Dr. Beppino C. Giovanella
Name/Address Stehlin Foundation
Performing Organization: Houston, TX

Contract Number: NO1-CB-33856
Starting Date: 2/1/73 Expiration Date: 1/31/78

Goal: To ascertain whether a thymusless-nude strain of Swiss mice can be used as transplant-recipient of human tumors for a study of their growth potential, hormone responsiveness, and their response to chemotherapeutic agents.

Approach: A colony of thymusless-nude Swiss mice has been developed and the good health of the animals permits their use in long-term experiments. Approximately 50 human mammary carcinomas will be transplanted each year. Each tumor sample is transplanted into at least 2 females and 2 males treated with AL(B)S, subcutaneously and under the kidney capsule. When enough material will be available, the tumor will be transplanted also in other nudes with the same modalities plus a source of angiogenetic factor(s). If the tumor grows, it will be retransplanted for at least 3 generations, if feasible. The transplanted tumor fragment will be characterized by a pathologist. The morphology and karyotype of the growing mass will be compared with the original fragment.

Progress: One hundred thirty-four primary and metastatic human breast carcinomas and 21 cell lines from human breast cancers have been transplanted in 1,470 nude-thymusless mice. Of 57 breast cancers transplanted directly from the patient into untreated nudes and observed for 90 days or more, 28 grew slowly. Four tumors showed indefinite growth. A fifth primary carcinoma grew rapidly, and has been transplanted serially. Eleven human breast cancer cell lines grew vigorously in the nudes. Two of these tumors metastasized to lymph nodes and lungs. Of 54 tumors transplanted into AL(B)S treated mice, 30 grew. Seven tumors so obtained, together with one which developed in untreated mice and eleven obtained injecting cultured cell lines, have already been successfully retransplanted at least once. Five serially transplanted human breast carcinomas have been tested for their sensitivity to various doses of 5-FU and adriamycin in 320 nudes. Three showed sensitivity to 5-FU, two of them were also sensitive to adriamycin, and two responded to neither drug. Two tumor cell lines grew faster in males and two in females.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 10/27/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $160,000

CONTRACT RESEARCH SUMMARY

Title: Purification of Estrogen Receptor in Tangible Amounts

Principal Investigator: Dr. Giovanni A. Puca
Name/Address Universita Di Napoli
Performing Organization: Napoli, Italy

Contract Number: NO1-CB-64074
Starting Date: 9/30/76 Expiration Date: 5/29/78

Goal: To isolate and purify estrogen receptor in milligram quantities.

Approach: Carry out large-scale tissue disintegration and centrifugation for isolation of estrogen receptor from calves' uteri. Use the sodium thiocyanate (NaSCN) technique for inhibition of aggregation and dissociation of receptor and purify the estrogen receptor by affinity chromatography. Furnish investigators, as recommended by the Breast Cancer Experimental Biology Committee, with pure receptor and its antibodies.

Progress: Calves' uteri have been obtained and frozen for future isolation and purification of estrogen receptor. Attempts at isolating the estrogen receptor has been hampered due to the delay in receiving the required instruments from the United States.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $70,000 (TQ)

CONTRACT RESEARCH SUMMARY

Title: Microcirculation and Molecular Transport in Mammary Carcinomas

Principal Investigator:	Dr. Joseph F. Gross	Dr. Marcos Intaglietta
Name/Address	University of Arizona	University of California
Performing Organization:	Tucson, AZ	San Diego, CA

Contract Number: NO1-CB-63981
Starting Date: 6/14/76 Expiration Date: 6/13/78

Goal: To study the local velocity and pressure in single vessels and quantitate the movement of materials across the endothelium of single vessels into the extravascular tissue in tumor microcirculation.

Approach: Fit Fischer 344 rats with a modified Algire access chamber containing the BL-DMBA mammary adenocarcinoma for analyses of microcirculatory parameters. Run similar studies using human mammary adenocarcinoma specimens with the Algire chamber implanted into nude-athymic mice. Obtain morphological data by planimetric techniques by measuring the diameter and length of developed two-dimensional microvasculature. Infuse intravenously fluorescein-tagged albumins and photograph the microvasculature through the image intensifier. Determine blood flow by measuring the velocity of red blood cells using the dual slit technique. Determine the capillary pressure by inserting a micropipette into the vessel. Characterize the diffusion and transport in the extravascular compartments by analyzing the extravasation of dyes injected intravenously or intra-arterially. Develop analytical models for characterizing microcirculation of tumor systems.

Progress: The Algire chamber was implanted in Fischer 344 rats. Mammary adenocarcinomas were implanted and analyzed during a 10-day growth period. The flow patterns in capillaries were quantitatively characterized through the measurement of the velocity of red blood cells in video tape recordings obtained with an image-intensified intravital television microscope. Observations were made over periods ranging up to one hour. It was found that there are two basic flow patterns, one steady and the other fluctuating periodically. Flow velocities are mostly low (0.2-0.5 mm sec). Combining the flow data with morphological data, it was found that the residence time of a red cell in the tumor microcirculation is long when compared with normal mesenteric and muscle microcirculation. The intent is to proceed in the near future using nude mice implanted with human tumors.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak, B.S., Richard Knazek, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/5/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: Dr. R. David Cole
Name/Address University of California
Performing Organization: Berkeley, CA

Contract Number: NO1-CB-43866
Starting Date: 6/29/74 Expiration Date: 6/28/77

Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones.

Approach: (1) Demonstrate hormone sensitivity in mammary epithelial cell line (NMuMg). (2) Assay for hormone receptors. (3) Observe the program of chromatin assembly through the sequence of appearance and modification of chromosomal proteins (e.g., histones and tubulin) and the effects of hormones on it. (4) Observe maturation of chromatin by characterizing 3 or 4 structural categories of chromatin fragments at different stages of chromatin maturation.

Progress: NMuMg cells grown in media containing charcoal-treated serum showed 2×10^4 steroid receptors and were induced to pronounced morphological changes by a combination of cortisol and prolactin (both were necessary). Although charcoal treatment seemed to give reproducible results for two months, subsequently the contractor was unable to maintain healthy NMuMg cells on charcoal-treated serum. Nevertheless, preliminary findings of hormonal responsiveness, even in cells maintained with untreated serum, were consolidated by more extensive, systematic studies. In particular, the two-fold stimulation of glyceraldehyde-3-phosphate dehydrogenase by insulin was firmly established, as was a 4-fold stimulation of lactose synthetase by insulin, cortisol and prolactin. It was proved that lactose is a product in this assay. The stimulation of lactose synthetase (A and B proteins) occurs as a wave about 13 days after plating cells in medium containing all three hormones.

Recently it was shown that in a single cell line (HeLa), H1 subspecies are not synthesized synchronously. This reveals a new aspect in the programming of chromatin assembly and may reflect the asynchronous synthesis of euchromatic DNA and heterochromatic DNA.

The competence of tubulin preparations for in vitro microtubule assembly has recently been found to depend on the degree of transformation of the cells from which they are isolated. Cells that have lost density-dependent, growth inhibition, and which grow in multiple layers yield the most competent preparations. Membrane-bound regulatory factors for assembly might be selectively lost in normal cells.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/13/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Cultures of Normal Mammary Epithelial Cells

Principal Investigator: Gordon H. Sato, Ph.D.
Name/Address University of California at San Diego
Performing Organization: La Jolla, California 92093

Contract Number: NO1-CB-74188
Starting Date: 7/1/77 Expiration Date: 6/30/78

Goal: To isolate and maintain growth of pure normal mammary epithelial cells in culture.

Approach: Determine the hormonal and nutritional requirements of established animal and human mammary tumor cell lines. Proceed with similar studies on primary cultures of human mammary tumors obtained from autopsies and mastectomies. Determine the hormonal content, blood serum components, and growth factors (ovarian, nerve, epidermal, etc.) to prepare a defined media to maintain growth and passage of normal mammary epithelial cell lines.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 4)

Project Officer: Chester V. Piczak Site Visit Date: None
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $55,700

CONTRACT RESEARCH SUMMARY

Title: Effects on Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator: Frank J. Talamantes, Jr., Ph.D.
Name/Address University of California at Santa Cruz
Performing Organization: Santa Cruz, California 95064

Contract Number: NO1-CB-74091
Starting Date: 7/17/77 Expiration Date: 7/16/78

Goal: Determine the effects of perinatal exposure to exogenous estrogens and progestins, singly and in combination, on mammary gland noduligenesis and tumorigenesis in female mice.

Approach: The hormonal agents, diethylstilbestrol, 17α-hydroxyprogesterone caproate, Ovral, ethynil estradiol, and norgestrel will be used singly and in combination for the studies. Prenatal and/or neonatal exposure to an estrogen and/or progestin will be done to determine whether mammary gland noduligenesis and tumorigenesis depends on: a) stage of gestation; b) dose and type of hormone; c) sensitivity of the mammary gland to oral contraceptives and sub-threshold and threshold doses of carcinogens; d) direct action of hormones on the gland or systemic factors; e) hormones in milk from lactating mother. Incidence, age of appearance and types of mammary dysplasias and tumors will be recorded. C_3H (MTV+,NIV+) and C_3Hf (MTV-,NIV+) female mice will be used for all studies.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 1 Approach 1)

Project Officer: Chester V. Piczak Site Visit Date: 4/22/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $91,200

CONTRACT RESEARCH SUMMARY

Title: Neoplastic Potential of Preneoplastic Lesions of the Human Mammary Gland

Principal Investigator: Dr. S. R. Wellings
Name/Address University of California
Performing Organization: Davis, CA

Contract Number: NO1-CB-43908
Starting Date: 6/28/74 Expiration Date: 6/27/77

Goal: Define the growth potential of hyperplastic-dysplastic lesions of the human mammary gland also termed "preneoplastic" or "latent cancer cells."

Approach: Analysis of the growth potential of heterotransplants of human mammary lobules and lobular lesions (atypical lobules, apocrine lobules, carcinoma in situ) and carcinomas of the breast in "nude" athymic mouse hosts. The lobules and lobular lesions are transplanted into host gland-free (cleared) mammary fat pads. Effects of the following are determined singly and in various combinations: collagenase pretreatment of lesions, prior in vitro cultivation, hormonal treatment of hosts (combinations of estrogen, progesterone, mammotropin, and pituitary isografts), and treatment of hosts with antilymphocyte serum (ALS). Animals are killed 4-24 weeks after transplantation and the transplants examined for survival, function, inflammatory response, vascular response, cytological dedifferentiation, growth, and outgrowth.

Progress: One hundred eighteen specimens of fresh human mammary tissue from 97 patients provided a total of 1,092 transplants into nude athymic mice. Most transplants were normal appearing lobules with lesser numbers of various atypical lobules and foci of carcinoma in situ. Enlargement and growth of transplants of lobules and atypical lobules were observed in several instances. Enlarging transplant tissue pushed into and against the mouse tissue in an expansile fashion but generally did not show invasive outgrowth of the kind observed with mouse mammary isografts. The relationship of growth and enlargement of transplants to pretreatment with collagenase, pituitary isografts, prior in vitro cultivation, hormonal treatment of host, and ALS treatment of host is unknown until all the results are analyzed. However, preliminary observations indicate that human mammary transplant tissue may be less responsive in a secretory sense to mouse pituitary isografts than the host's own gland. Further evidence suggests that treatment of the host animal with estrogen plus progesterone plus mouse pituitary isografts may encourage active growth and expansion of both epithelial and stromal components of the transplanted tissue. This last finding is important since it strongly suggests that the host gland-free fat pad of the nude athymic mouse may be a suitable transplant site for human mammary tissue in studies of growth potential.

Significance for Cancer Research (NCP Objective 4,5 Approach 2,4)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Date Visit: 3/12/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biochemical Nature of Hormone Dependency in Breast Cancer

Principal Investigator: Dr. Eugene DeSombre
Name/Address
Performing Organization: University of Chicago
Chicago, IL

Contract Number: NO1-CB-23873
Starting Date: 6/1/72 Expiration Date: 11/30/77

Goal: To assess the role of hormones and specific hormonal receptors in the control of tumor growth.

Approach: Carcinogen-induced (DMBA) primary or transplantable mammary tumors of rats and appropriate control tissues are utilized. Major emphasis is to be placed on tumor RNA polymerase activity as affected by hormones; however, other projects on hormonal control of tumor growth and the role of hormonal receptors are to be continued. Major areas of study are tumor RNA polymerase (I and II) activity; hormone receptors in apparently autonomous mammary tumors; interrelation of estrogen and prolactin receptors in tumors; transplantable, hormone-dependent mammary tumors; and regulation of tumor peroxidase activity.

Progress: Before assaying for RNA polymerase stimulation by estrogen the tumor is classified as hormone-dependent (HD) or independent (HI) according to in vivo tumor response following castration. Assay is in a low salt Mg^{++} system, is dependent on all 4 ribonucleotide precursors, and is inhibited by actinomycin D. At 30°C RNA synthesis is linear for 5-10 minutes with no appreciable difference in product degradation in hormone-treated and control groups. Incubation of tumor homogenates with estradiol (E2) stimulates RNA polymerase I activity in hormone-dependent tumors but not with hormone-independent tumors. Requirements for E2 stimulation are receptor containing cytosol and HD tumor nuclei. Anti-estrogen (CI628) which effects regression of HD tumors in vivo appears to block E2-induced stimulation in vitro. Some but not all HI tumors have significant concentrations of estrogen receptors. Mammary tumor peroxidase, characteristic of HD tumors, disappears after ovariectomy but reappears after E2 administration in vivo. Subcellular distribution of peroxidase is characteristic of tumor cell synthesis rather than cell uptake. Correlation of tumor concentrations of both prolactin and estrogen receptors shows most HD tumors have high levels of both receptors and HI tumors generally have low levels of both. Some HD tumors have elevated levels of only one receptor, implying a spectrum of dependences, some on both hormones, some on only one of the two hormones.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/12/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Effect of Mammary Carcinoma Cells on the Vascular Endothelium

Principal Investigator: Richard R. Lindquist, Ph.D.
Name/Address University of Connecticut
Performing Organization: Farmington, Connecticut 06032

Contract Number: NO1-CB-74089
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To elucidate the cancer cell-endothelium interaction and the effect cancer cells have on the vascular endothelium.

Approach: Human mammary cancer cells in co-culture with monolayer endothelial cells will be the model in studies to determine: (a) cancer cell-endothelial interactions using time-lapse phase contrast microscopy and electron microscopy; (b) interacting forces involved; (c) influence of clotting, polymorphonuclear leukocytes, platelets, microfilaments, microtubules, c-AMP on strength of the interactions; (d) cancer cell inflicted damage to endothelium; (e) cancer cell induced directional proliferation of endothelial cells. Human cells will be obtained from the Cell Bank (BCTF) and endothelial cells will be isolated from umbilical cords obtained from local hospitals.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 3)

Project Officer: Chester V. Piczak Site Visit Date: 4/21/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $84,200

CONTRACT RESEARCH SUMMARY

Title: Osteotropism of Mammary Carcinoma Metastasis

Principal Investigator: Dr. Gregory R. Mundy
Name/Address University of Connecticut Health Center
Performing Organization: Farmington, CT

Contract Number: NO1-CB-63980
Starting Date: 6/21/76 Expiration Date: 6/20/78

Goal: To identify the mechanisms by which bone is altered in breast cancer.

Approach: (1) Determine the cellular mechanism of bone destruction in metastatic breast cancer. (2) Determine the hormonal factors responsible for hypercalcemia and/or bone metastasis in breast cancer. (3) Characterize the effects of these factors on bone and identify ways in which those effects can be prevented or opposed. (4) Investigate the production of substances which stimulate new bone formation and collagen synthesis in breast cancer. In vitro and in vivo systems of human and animal models are utilized.

Progress: Different types of neoplastic cells were surveyed for bone resorption activity. The contractor has evaluated seven different human breast cancer cell lines, and NMU-induced mammary carcinoma in rats and human ovarian carcinoma and bronchogenic carcinoma which were derived from patients with hypercalcemia. Current studies involve identification of bone resorption factors in those neoplastic processes revealing positive results in their assay system.

Monocytes and macrophages have been found to produce bone resorption. The contractor is attempting to elucidate the mechanisms involved and the relationship of these findings to neoplasia-bone interaction.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Chester V. Piczak, B.S., David Young, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/19/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $119,100

CONTRACT RESEARCH SUMMARY

Title: Structure Function Relationship of Prolactin Interactions in Mammary Gland Cells

Principal Investigator: Dr. Kurt E. Ebner
Name/Address University of Kansas Medical Center
Performing Organization: Kansas City, KS

Contract Number: NO1-CB-63985
Starting Date: 6/14/76 Expiration Date: 6/13/78

Goal: To modify ovine prolactin and determine the critical amino acids involved in binding of the hormones to membranes, activation of cyclases or kinase and stimulation of α-lactalbumin formation.

Approach: Modify prolactin chemically by using mercaptoethanol, N-ethylmaleimide, iodoacetamide, cyanogen bromide, tetranitromethane, and carbodiimide. Use trypsin, chymotrypsin, plasmin, pepsin, and leucine amino acid peptidase for enzyme modification of prolactin. Modifications will include: (tyrosin, tryptophan, lysine, methionine, histidine), carboxyl groups and reduction of disulfides. Prolactin activity will be assayed by binding to mammary particles, ability to activate cyclases or protein kinase in mammary plasma membranes and stimulate α-lactalbumin synthesis in mid-pregnant mammary explants.

Progress: Mouse α-lactalbumin has been purified and antibodies are being raised in order to develop a RIA for this protein. About 50 mg of rat α-lactalbumin have been produced and antibodies are being produced. Attempts to modify prolactin (PRL) chemically have centered on the 3 disulfide bonds. Reduction and alkylation of these bonds result in 80% loss of binding activity of the molecule. Reduction alone results in a 75% loss of binding activity, indicating that the integrity of the disulfides is important but not absolutely essential.

Chemical modification of PRL will continue with emphasis on the disulfides and small fragments produced by limited proteolysis. These derivatives of PRL will be tested for biological as well as binding activity.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/2/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $91,300

CONTRACT RESEARCH SUMMARY

Title: Study of Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: Dr. William H. Pearlman
Name/Address Performing Organization: University of North Carolina School of Medicine
Chapel Hill, NC

Contract Number: NO1-CB-53905
Starting Date: 4/1/75 Expiration Date: 3/31/78

Goal: (1) To detect, characterize, and quantify steroid hormone-binding proteins in the whey of human pre- and postpartum colostrum, human milk, and animal milk. (2) To determine the influence of multiple endogenous and exogenous hormones on whey levels of steroid-binding proteins.

Approach: (1) Quantitative assay of steroid hormone-binding activity (tritium-labeled progesterone, cortisol and other glucocorticoids, 17β-estradiol, and testosterone) in whey. Physicochemical characterization (elution behavior on Sephadex gel and ion-exchange chromatography, equilibrium association constants, steroid ligand specificity). Comparison of whey with serum steroid-binding proteins. (2) Correlation of changing endocrine status (i.e., known alterations in multiple hormone levels during advancing pregnancy, parturient and postpartum periods) with changing levels of steroid-binding proteins in whey and coincidental serum. Studies, similarly, following the administration of lactation-suppressing agents, particularly estrogen, in humans and rats, and of lactation-stimulating agents, particularly glucocorticoids in rats.

Progress: Corticosteroid-binding proteins (CBP) were detected in the whey of human colostrum and milk and rat milk which resemble serum corticosteroid-binding globulin (CBG) in certain respects. The CBP level is appreciable during the prepartum period in humans; it declines precipitously at parturition. The level of CBP in rat milk is substantial, i.e., about 25% of that of serum CBG. The rat would appear to be a suitable animal model for further study. Physiological significance may be attached to a progesterone- and cortisol-binding protein which appears in appreciable amounts in human prepartum colostrum. This protein may regulate the local concentration of the bound and unbound forms of these hormones in a fluid which bathes the epithelial cells lining the mammary acini and ducts. It is also conceivable that serum CBG facilitates the transport of cortisol and progesterone into the mammary epithelial cell. Further studies are in progress which indicate that endogenous and exogenous hormones influence markedly the levels of whey CBP and coincidental serum CBG.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 7/24/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Methylation of Transfer RNA in Normal and Neoplastic Mammary Tissue

Principal Investigator: Phoebe S. Leboy, Ph.D.
Name/Address University of Pennsylvania
Performing Organization: Philadelphia, Pennsylvania 19174

Contract Number: NO1-CB-74108
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To examine tRNA methylation in normal mammary gland and DMBA-induced hormone responsive and non responsive tumors in the rat.

Approach: Compare the level of methyltransferase activity in normal quiescent, late pregnant and lactating mammary tissue and DMBA-induced mammary tumors. Examine patterns of tRNA methyltransferase activities in hormone responsive and non responsive tumors. Purify individual tRNA methyltransferases from mammary tumors and corresponding enzymes from normal mammary gland and compare their biochemical properties. Use purified tRNA for screening of potential methyltransferase inhibitors and test the inhibitors in vitro and in vivo to determine the effect on cell or tumor growth.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: Chester V. Piczak Site Visit Date: 4/18/77
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: Approx. $100,000

CONTRACT RESEARCH SUMMARY

Title: Studies of Isoenzymes in Normal, Preneoplastic and Neoplastic Breast Tissues

Principal Investigator: Dr. Russell Hilf
Name/Address University of Rochester
Performing Organization: Rochester, NY

Contract Number: NO1-CB-33880
Starting Date: 5/21/73 Expiration Date: 12/20/76

Goal: To examine multiple molecular forms of glucose 6-phosphate dehydrogenase (G6PD) and lactate dehydrogenase (LDH) to characterize normal, preneoplastic and neoplastic mouse mammary tissue.

Approach: Molecular forms of G6PD and LDH to be examined in normal mouse mammary tissue (pregnancy, lactation, parturition) and in preneoplastic and neoplastic tissue exposed to the same hormonal milieu in vivo. Determine sulfhydryl content of tissues, with emphasis on reduced glutathione, to seek correlations with G6PD profiles in normal and abnormal tissue. Establish the molecular weights of G6PD-III, II and I, using methods such as that of Hedrick-Smith. Purify and characterize G6PD from glands of lactating mice. Use G6PD as an antigen to induce antibodies. Expand studies on G6PD forms in tissues of mice possessing NIV and MTV.

Progress: Glutathione reductase (GR) activity was elevated in HAN and tumors compared to mammary glands from pregnant or lactating mice. The contractor developed methods for quantitation of GSH (the product of GR activity) and GSSG, the oxidized substrate for GR. On both a tissue weight and mg protein basis, levels of GSH were elevated (~2-fold) in HAN and tumors from virgin or lactating mice compared to levels of GSH in mammary glands from pregnant or lactating animals. GSSG was similar in all tissues. These data support the earlier data in tissues with increased GR activity. This finding demonstrates a biochemical alteration shared equally by HAN and tumor and significantly different from normal tissue. Regenerating liver (normal rapidly proliferating tissue) showed no elevation in GSH levels; effects seen with abnormal tissues are not solely due to rapid proliferation. Preliminary sampling of human tissues showed significantly elevated GSH levels in carcinomas. G6PD forms (Hedrick-Smith method) separate after electrophoresis due to size and not charge; elution of each form and re-electrophoresis demonstrated that each form migrated as originally separated, indicating that no aggregation had occurred in the gels at the site of enzyme localization. Molecular weight estimates were: G6PD-III, 215,000; G6PD-II, 100,000; G6PD-I, 55,000 indicating a tetramer:dimer:monomer relationship. G6PD-III required more antibody (anti-G6PD) for inactivation than G6PD-II indicating that fewer antigenic determinants were accessible in the multimeric form of the enzyme.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 6/21/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine-Induced Breast Cancer Regression

Principal Investigator: Dr. William L. McGuire
Name/Address University of Texas Health Science Center
Performing Organization: San Antonio, TX

Contract Number: NO1-CB-23862
Starting Date: 6/1/72 Expiration Date: 5/31/78

Goal: To obtain new information of the hormonal control of breast tumor growth and to identify those breast cancer patients whose disease will respond to hormonal manipulation.

Approach: Investigate the sequence of events from initial estrogen binding to receptor sites to nuclear localization of receptor complex and estrogen stimulation of progesterone receptor synthesis.

Progress: Approximately 150 human breast tumors have been assayed for cytoplasmic receptor (ER) and evaluated for clinical response to endocrine therapies. Tumors without ER have a low response rate (10%) whereas ER-positive tumors had 60% objective regression. Tumors with very high ER values had the highest remission rate.

New assays currently being developed to measure nuclear bound ER. Previous efforts unsuccessful because of nuclear protease activity which promptly degrades nuclear ER. Preliminary results with new assay avoids protease problem. An unexpected finding of potential major significance is of nuclear ER without attached steroid in certain tumors. The contractor speculates that uncharged nuclear ER may be stimulating replication, perhaps explaining lack of tumor regression following ablative procedures. Antiestrogens may be the choice of therapy in these special tumors.

Progesterone receptor (PgR) synthesis was chosen as a new biochemical marker of an intact estrogen response system. Estrogen priming in DMBA rat mammary tumors and MCF-7 human breast cancer cell lines results in dramatic induction of PgR. In metastatic human breast tumors the presence of PgR directly correlates with the level of ER. In 44 patients ER and PgR correlate well with response to therapy. ER-, PgR-, 0%; ER-, PgR+ no patients; ER+, PgR- 27%; ER+, PgR+ 76%. The presence of PgR in ER+ tumors gives considerable confidence in predicting response to therapy.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/24/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $125,000

CONTRACT RESEARCH SUMMARY

Title: Alteration of Mammary Neoplastic Cells by Manipulation of Cellular Environment

Principal Investigator: Dr. Creed W. Abell
Name/Address University of Texas Medical Branch
Performing Organization: Galveston, TX

Contract Number: NO1-CB-43906
Starting Date: 6/10/74 Expiration Date: 6/9/78

Goal: To manipulate the cellular environment in vivo and in vitro in order to limit preferentially the growth of neoplastic cells of mammary origin.

Approach: To evaluate the effects of phenylalanine ammonia-lyase (PAL) and phenylalanine restriction on mammary tumors in mice and rats and on human breast tumors growing in athymic mice. Several PALs, obtained from different sources or modified chemically to decrease their rates of clearance from the host, will be studied and compared for therapeutic effectiveness.

Progress: PAL, isolated from the yeast Rhodotorula glutinis, has been purified to homogeneity and evaluated, alone and in combination with phenylalanine mustard (PAM), against BW10232 and 15091A mammary tumors in mice. PAL inhibited tumor growth for 5-10 days while PAL plus PAM was more effective than either agent alone. Repeated injections of PAL, however, resulted in the formation of antibodies (demonstrated by passive hemagglutination) that rapidly removed the enzyme from the circulation. Attempts to induce immunosuppression (tolerance) to PAL have been initiated with radiation or cyclophosphamide. Sublethal total-body radiation allowed relatively constant initial enzyme activities to be maintained in plasma after repeated PAL administration and markedly delayed (for 10-15 days) the accelerated rate of clearance of PAL. Under certain experimental conditions, cyclophosphamide selectively suppresses anti-PAL formation. Based on these findings, the therapeutic evaluation of PAL in immunosuppressed tumor-bearing mice and rats is currently ongoing.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/14/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $85,000

CONTRACT RESEARCH SUMMARY

Title: Differentiation of Mammary Epithelial Cells

Principal Investigator: Dr. Howard L. Hosick
Name/Address Washington State University
Performing Organization: Pullman, WA

Contract Number: NO1-CB-63986
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To study how several mesenchymal cell types in the adult mouse mammary gland interact with mammary epithelium to regulate its development.

Approach: Isolate mammary epithelial cells from C3H mice in midpregnant state. Grow these cells as monolayers in culture dishes and on gas-permeable substrata. Test the responses of the epithelium, under various endocrine conditions, to other mammary cell types and their products. The emphasis is on interactions with adipose cells and lipids. Growth responses of epithelial cells to fatty acids will be measured and metabolism of exogenously supplied lipids will be analyzed. Responses of normal, preneoplastic, and neoplastic cells will be compared. Undertake studies for defining conditions for growth and passage of primary cultures of normal epithelial cells.

Progress: Unilamellar liposomes containing free fatty acids have been prepared. These lipids are taken up by mammary cells in monolayers and form droplets in the cytoplasm. The rate of uptake by tumor cells is about twice that by normal cells. Under proper culture conditions, liposomes stimulate growth of the cells. Tumor cells are more sensitive to lack of lipid in the medium. Problems of fibroblasts overgrowing the cultures and proper growth conditions for normal and malignant cells are being dealt with. Further work will attempt to modify liposome-induced growth response with various hormones and with mesenchyme. Free fatty acids from cultured mammary fat pads will also be tested. The metabolism of the exogenously added lipids will be examined.

Significance for Cancer Research (NCP 3 Approach 3,5)

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/4/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $53,600

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of Mammary Cell Surface

Principal Investigator: Dr. Clayton Buck
Name/Address Wistar Institute
Performing Organization: Philadelphia, PA

Contract Number: NO1-CB-64069
Starting Date: 6/28/76 Expiration Date: 6/27/78

Goal: To determine the synthesis and fate of mammary tumor glycoproteins.

Approach: Glycoproteins will be derived from mouse mammary tumor systems and normal mammary tissue labeled *in situ* or following growth in culture with ^{3}H- or ^{14}C-labeled sugars. Future studies will use human mammary cell lines from the Cell Culture Bank and surgical specimens. Membrane-bound and shed glycoproteins will be analyzed by polyacrylamide gel electrophoresis. Antisera will be prepared against material shed from tumor and control cells. The antisera will be used to identify and isolate membrane glycoproteins shed from tumor cells. Lectin and immunoglobulin affinity columns will be utilized in the purification of glycoproteins. Turnover of specific membrane glycoproteins will be followed using specific antisera and affinity chromatography.

Progress: The following cell lines were established: MG (normal mammary cell), MT_2 (non-virus tumor line), MT_3 (virus producing tumor line), two human cell lines and primary cultures from mid-pregnant mice. Comparative studies of pronase digests of tryptic glycopeptides from MG, MT_2, and MT_3 cell cultures were done. Glycopeptides from MT_2 tumor are more complex than those from MT_3 tumor. MT_2 tryptic glycopeptides yield the largest glycopeptides after pronase digestion. The glycopeptides are heterogeneous and have molecular weights ranging from 3,600 to greater than 4,800 daltons. The MT_3 profile is less complex with a majority of glycopeptides in the 3,600 to 4,800 dalton range. The tryptic glycopeptides from normal cell line (MG) are similar to the MT_3 tumor cell line.

MT_2 and MT_3 glycopeptides derived from the cell culture medium appear more homogeneous than those derived from the cell surface treated with trypsin. Glycoproteins shed from the cell are not the result of random degradation and turnover of membrane glycoproteins. The shed and membrane bound glycopeptides from the MG normal cell line are not identical.

Production of antisera to glycoproteins shed into the cell culture media from mammary tumor and normal cells has begun. Supernates containing all of the material shed into serum-free tissue culture medium over a 24-hour period are injected into rabbits. The antisera produced in the rabbits will be evaluated.

Significance for Cancer Research (NCP Objective 5 Approach 3,4)

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/27/76
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $99,700

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Mammary Epithelial Cell Plasma Membrane

Principal Investigator: Dr. Grant Fairbanks
Name/Address Worcester Foundation for Experimental Biology
Performing Organization: Shrewsbury, MA

Contract Number: NO1-CB-33908
Starting Date: 6/1/73 Expiration Date: 2/28/78

Goal: Development of new methods for plasma membrane isolation and characterization applicable to normal and neoplastic mammary cells.

Approach: Novel radiolabeled reagents and activated beads will be prepared and used to selectively modify cell surfaces. The altered physical or chemical character of plasma membranes from the modified cells will be the basis for membrane purification. Plasma membranes from normal and malignant human mammary cells maintained in monolayer cultures will be characterized.

Progress: In the first radiosynthesis of a cleavable protein cross-linking reagent, ^{35}S-dithiobis(succinimidyl propionate) (^{35}S-DTSP) was prepared at 20 mCi/mmole. DTSP is unique in combining high reactivity with long lifetime in aqueous solution. Protein amino groups are cross-linked with high efficiency under mild conditions: 1-5 minutes at 0°, pH 7, with 10^{-4}-10^{-3} M DTSP. Ascites cells of the 13762 rat mammary adenocarcinoma are rapidly and reversibly cross-linked by DTSP. Homogenization of DTSP-treated cells in hypotonic buffer is being studied as an approach to the rapid isolation of plasma membrane fragments in which specific associations with extracellular and cytoplasmic elements are preserved by cross-linking.

DTSP is also used in the sequential assembly of asymmetric reagents and activated beads. Pyridoxamine phosphate was condensed with ^{35}S-DTSP to yield a new nonpermeating membrane labeling reagent. Plasma membranes from cells derivatized with the reagent will be purified by selective adsorbtion on immobilized apo-aspartate aminotransferase.

The surfaces of polyacrylamide and glass beads have been polylysinated to greatly increase capacity and stability, and to minimize interference by excess unutilized internal sites. Small beads activated by reaction with DTSP are coupled to cell surfaces for direct membrane isolation. Larger beads, similarly activated, are used to immobilize selective adsorbents.

The spontaneous binding of ^{3}H-poly(U) to 13762 tumor cells has been studied. Poly(U) binding was found to be mediated by surface protein receptors with specificity for uridine, and to be modified by growth or incubation in vitro.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $60,000

CONTRACT RESEARCH SUMMARY

Title: Role of 3',5'-cAMP and Related Enzyme Systems in Mammary Neoplasia

Principal Investigator: Dr. David Maudsley
Name/Address Worcester Foundation for Experimental Biology
Performing Organization: Shrewsbury, MA

Contract Number: NO1-CB-33919
Starting Date: 6/1/73 Expiration Date: 11/30/76

Goal: To study the role of cyclic adenosine 3',5'-monophosphate (cAMP) in the mammary gland tissue, normal and abnormal, and to establish whether the incidence of mammary tumors alters cAMP metabolism.

Approach: The initial studies used freshly isolated cell suspensions and primary monolayer cultures of mouse epithelial cells, but present studies are concerned with comparing the findings obtained with the mouse tissues with those of established human cell lines. The effects of prostaglandins and prostaglandin precursors on cAMP production will be studied. Procedures for manipulating endogenous levels of cAMP and observing the subsequent effects on mammary cell growth are being developed.

Progress: Arachidonic acid (AA) and prostaglandin E_1 (PGE_1) produced a transient increase in cyclic AMP levels in primary monolayer cultures of C3H mouse mammary tumor epithelial cells. Naproxen, an inhibitor of prostaglandin synthetase, inhibits the effect of AA on cAMP levels but not that of PGE_1 epinephrine or cholera enterotoxin. A comparison between the effects of AA on cyclic AMP levels and prostaglandin production indicates that maximum cyclic AMP levels are observed prior to the peak in prostaglandin levels. These studies have shown that the effect of AA is mediated through a metabolite, but its identity with a prostaglandin has not been established.

Comparison of cyclic AMP metabolic profiles between rat and mouse tumors and a human cell line HBL-100 indicates significant differences between them. In HBL-100 cells cyclic AMP levels are increased by PGE_1 to a much greater extent than by epinephrine, whereas the reverse is observed in C3H mouse cells and rat DMBA, 13762 and R-35 tumor cells. Adenyl cyclase in HBL-100 is also poorly responsive to cholera enterotoxin, again in contrast to the rodent tumor cells, suggesting that there may be a deficiency in membrane GM_1 ganglioside, the binding receptor for enterotoxin in the human cell line.

Significance for Cancer Research (NCP Objective 3 Approach 3)

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Dr. Lewis M. Schiffer
Name/Address Allegheny General Hospital
Performing Organization: Pittsburgh, PA

Contract Number: NO1-CB-43899
Starting Date: 2/1/74 Expiration Date: 1/31/78

Goal: To develop and exploit methods of rapidly determining cell kinetic parameters of individual human breast cancers so that this information may potentially be used in the study of the natural history and treatment of these tumors.

Approach: Three cell kinetic parameters of human and non-human solid breast tumors are measured directly by tumor imprints and mechanical disaggregation of tissue followed by *in vitro* incubation with isotopically labeled thymidine. These parameters are ^{3}HTdR labeling index, DNA synthesis time, and PDP index (which estimates growth fraction). Potential doubling time and cell cycle time can be calculated from these values. The cell kinetics of human breast tumors, both primary and metastatic, are being collated and correlations with clinical findings attempted. After the acquisition of a sufficient data base, the perturbations of human tumor cell kinetics by therapeutic modalities will be studied. Likewise, perturbations of normal kinetics following single and multiple drug chemotherapy are being utilized to time-sequence additional therapy. The C3H spontaneous and 13762 transplantable mammary tumors are being utilized for this purpose.

Progress: The cell kinetics of the C3H tumor have been characterized. A number of correlations can be made, including direct relationship of tumor doubling time with cell cycle time and potential doubling time, and indirect relationship of tumor doubling time and cells in DNA synthesis. Studies with the 13762 tumor show cures of large tumors using cell kinetic predicted optimum time-sequencing, whereas predicted bad times showed poor results. The kinetics of human primary and metastatic tumors were different, with the latter showing ^{3}HTdR LI's double that of the primary and cell cycle times that were quite rapid. Correlations with clinical data are still in progress but ^{3}HTdR LI and PDP indices show indirect relationships with tumor volume. Results can be made available in seven days.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $173,100

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Lewis M. Schiffer, M.D.
Name/Address Allegheny General Hospital
Performing Organization: Pittsburgh, Pennsylvania

Contract Number: NO1-CB-75083
Starting Date: 7/1/77 Expiration Date: 6/30/78

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: The experiments on two animal tumor models, the DMBA induced rat mammary tumor and the C3H/He mouse spontaneous mannary tumor, will test the correlation of unperturbed cell kinetics and tumor response in individual animals to chemotherapeutic agents. The cell kinetic studies will include tritiated thymidine labeling index, DNA synthesis time and tumor growth fraction estimation via primer-available DNA polymerase assay. The experiments will be carried out on animals with primary tumors, on animals with primary tumors and metastases, and on animals with metastasis in which the primary has been removed. Tumors will be followed in the animals to determine doubling time, then bisected or subjected to needle biopsy for cell kinetic measurements. The animals will be treated with a drug for measurement of chemosensitivity, the drug response evaluated and correlated with cell kinetic parameters. The test drugs will be cyclophosphamide, methotrexate, fluorouracil, phenylalanine mustard and prednisolone.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment Site Visit Date: 1/26/77
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $98,000

CONTRACT RESEARCH SUMMARY

Title: Therapy for Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Dr. Charles A. Hubay
Name/Address Case Western Reserve University
Performing Organization: Cleveland, OH

Contract Number: NO-CB-43990
Starting Date: 6/16/74 Expiration Date: 6/15/78

Goal: To test the effects of adding chemotherapy, anti-estrogen therapy, and BCG to mastectomy in patients with locally advanced breast cancer (Stage II-III).

Approach: Patients under age 76 who show axillary nodes involved with metastases at the time of surgery are eligible for this study. Stratification, but not treatment selection, is on the basis of the presence or absence of estrogen receptor protein (ER) in the tumor. Random treatment assignments are (1) cyclophosphamide, methotrexate, 5-fluorouracil (CMF); (2) cyclophosphamide, methotrexate, 5-fluorouracil, tamoxifen (CMFT) for 12 months, and (3) CMFT for 12 months plus BCG for 12 months. Endpoint is the first evidence of treatment failure, i.e., the appearance of locally recurrent or of distant tumor.

Progress: This study began in September 1974, and more than 125 patients have been entered. Accession was faster after the requirement was dropped to 1 or more positive nodes, and rapidly accelerated after the control group was eliminated and substituted by CMF treatment. Insufficient time has elapsed to permit meaningful data about treatment failure of survival. Only six patients have voluntarily withdrawn from the study because of intolerance to treatment. No patient has shown irreversible toxicity.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 4/1/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $218,800

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Dr. Edward F. Scanlon
Name/Address Evanston Hospital
Performing Organization: Evanston, IL

Contract Number: NO1-CB-53917
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To determine the effects of adding chemotherapy and BCG to mastectomy for patients with locally advanced breast cancer.

Approach: Patients who have metastatic axillary nodes or Stage III breast cancers are admitted to the study program. Stratification and randomization are carried out along with a balancing for prognostic factors according to the following variables: Primary tumor size, number of positive nodes, menstrual status, and unfavorable local signs. The statistical analysis involves sequential treatment assignment and is designed to provide the greatest balance between all groups assigned according to all available prognostic indicators. One of three treatment schedules is assigned to each of the patients in the study according to the above computer classifications: (1) Intermittent phenylalanine mustard (PAM); (2) 5-fluorouracil, cyclophosphamide and prednisone in intermittent courses; and (3) the three-drug chemotherapy plus BCG inoculations. Two of the three treatment schedules are identical with the Mayo Clinic adjuvant study and the data will be cumulative. The statistical method of analysis is also identical with that now being used at the Mayo Clinic. The treatment courses will be given for one year or until there is evidence for relapse, whichever occurs first. Immunologic evaluation is done initially and every three months. Survival and recurrence-free intervals will be computed for each treatment group.

Progress: Ninety-one patients were enrolled through April 1977; 51% had tumors greater than 3 cm; 22 had unfavorable local signs; 53% had fewer than 4 axillary lymph nodes positive; 19% were premenopausal. Nausea has been the most common side effect, followed by hair loss. Relatively few blood changes have been seen. Metastases have become apparent in six patients, 16 to 68 weeks following mastectomy.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Donald Henson, M.D.
Program: Breast Cancer Treatment Site Visit Date: 8/29/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $99,900

CONTRACT RESEARCH SUMMARY

Title: Surgical Adjuvant Chemotherapy in Patients with Breast Cancer

Principal Investigator: Dr. David L. Ahmann
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-33899
Starting Date: 6/30/73 Expiration Date: 6/29/78

Goal: To assess the effects of surgical adjuvant chemotherapy utilizing a multiple drug program: cyclophosphamide, 5-fluorouracil and prednisone (CFP), versus CFP plus irradiation therapy versus a program utilizing phenylalanine mustard alone.

Approach: Patients with unfavorable primary breast cancers (node +) are assigned to treatment programs involving mastectomy followed by multiple drug chemotherapy (CFP), multiple drug chemotherapy (CFP) with postoperative irradiation, or single agent chemotherapy utilizing phenylalanine mustard. After stratification according to menstrual category, tumor size and degree of involvement, each patient is randomly assigned to one of the three treatment programs as indicated above. The adjuvant chemotherapy is initiated two to four weeks postoperatively and given concomitantly with radiation therapy when this modality also is employed. Chemotherapy is terminated at the end of one year (10 courses) or upon the appearance of recurrent disease, whichever occurs first.

Progress: Patient entry in the study began in September 1973. As of April 1977, a total of 159 patients had been entered on study, 153 thus far evaluable. There have been 4/8 recurrences in patients receiving a former treatment which involved radical mastectomy alone and which was subsequently abandoned. There have been 10/42 recurrences in the phenylalanine mustard treatment group thus far at 16 to 98 weeks. Combination chemotherapy program CFP has been associated with recurrences in 10 of 49 evaluable patients (4 recurrences became evident following cessation of chemotherapy). Of 40 evaluable patients who received CFP and radiation therapy, 8 have shown recurrent cancer thus far and these were at 7 to 94 weeks following completion of the chemotherapeutic program. There have been no drug deaths. Myelosuppression is a usual accompaniment to the cytotoxic chemotherapy programs; it seems to be least severe with phenylalanine mustard and most pronounced with the addition of radiation therapy to the CFP programs. Continued statistical analysis is an ongoing part of this project.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $94,850

CONTRACT RESEARCH SUMMARY

Title: Predictive Transplantable Animal Mammary Tumor Models

Principal Investigator: Dr. Arthur E. Bogden
Name/Address Mason Research Institute
Performing Organization: Worcester, MA

Contract Number: NO1-CB-43914
Starting Date: 11/1/73 Expiration Date: 10/31/77

Goal: To carry out preclinical evaluations of single and combined therapeutic modalities in experimental mammary tumors of known biological characteristics.

Approach: Studies are being done in six transplantable rat mammary tumor systems having a spectrum of histological, growth and metastasizing characteristics. The highly metastatic 13762 mammary adenocarcinoma is used most extensively and the R3230AC, SMT-2A, 3M2N, DMBA#1 and DMBA#14 mammary tumors are used to further test and evaluate those drug and therapy combinations showing promising activity in the 13762 system. Chemotherapy assays versus subcutaneous grafts are made by initiating drug treatment on the day following implantation or on day 15 when tumors are well established. Metastasis assays combine surgical extirpation of tumors with the modalities of chemotherapy, x-irradiation and immunotherapy, alone and in various combinations. The parameters of immunosuppression and hormone radioimmunoassay are applied in studies requiring in-depth drug and therapy evaluation. Chemotherapeutic agents of current interest include phenylalanine mustard (PAM), adriamycin (ADR), cytoxan (CTX), 5-fluorouracil (5-FU), methotrexate (MTX), dibromodulcitol (DBD), hexamethylmelamine (HMM), vincristine (VIN), and the nitrosoureas.

Progress: Studies with the 13762 mammary adenocarcinoma predictive for those human breast cancers susceptible to the alkylating agents have shown that (a) optimal time for crossover from CTX to PAM was the period of maximum remission, cross resistance becoming evident and more pronounced the longer substitution was delayed; (b) the more effective agent, PAM, was more effective alone than initiating therapy with a less effective agent, CTX, and then crossing over to PAM in rotation sequence; (c) crossing over from CTX to DBD was superior as compared to CTX alone or crossovers to PAM or HMM, HMM being least effective. The combination of PAM + 5FU was synergistic in terms of remission induction and maintenance as well as increased life span, in both PAM very responsive and only moderately responsive mammary tumor systems. The administration of PAM, CTX, DBD and HMM to normal intact and ovariectomized female rats significantly increased serum estrogen levels.

Significance for Cancer Research (NCP Objective 6C Approach 1)

Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Treatment Site Visit Date: 3/22/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $169,500

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Arthur E. Bogden, Ph.D
Name/Address Mason Research Institute
Performing Organization: Worcester, Massachusetts

Contract Number: NO1-CB-74208
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual test cancers.

Approach: The test system in this project is the subcapsular renal implant. Five rodent model tumor systems will be used first in syngeneic mice and then in nude athymic mice. After the growth characteristics of the implanted tumors are determined, the effects of chemotherapeutic agents administered to the host animals will be studied. The test drugs will be cyclophosphamide, methotrexate, fluorouracil, phenylalanine mustard, vincristine and adriamycin. The effects of the drugs on the tumor implants will be determined by comparing their growth characteristics in drug-treated animals with those in unteated animals. If the animal tumor systems demonstrate that the subcapsular renal implant method is feasible and produces reproducible results, its usefulness for studies on human breast cancers will be explored.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)

Project Officer: Mary E. Sears M.D.
Program: Breast Cancer Treatment Site Visit Date: 1/24/77
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $80,500

CONTRACT RESEARCH SUMMARY

Title: Biomedical Computing Software Services

Principal Investigator:	Anne Kramer
Name/Address	Mason Research Institute
Performing Organization:	Worcester, MA

Contract Number: NO1-CB-74140
Starting Date: 3/21/77 Expiration Date: 3/20/78

Goal: To increase the usefulness of data produced in projects related to the treatment of human breast cancer.

Approach: A central data file will be set up for three areas of study: (1) surgical adjuvant therapy, (2) tumor markers, and (3) estrogen receptor assays. The file will allow comparison of the results from various studies and provide a data base from which material can be quickly and conveniently retrieved when desired for testing new ideas, identifying groups of patients suitable for more detailed study, and for preparing reports to the medical and general public. The file is not intended to duplicate those at individual institutions but to perform analyses that are not possible at the individual institutions.

Progress: Mason personnel have designed a data collection and editing system for the breast cancer studies data center. Clinical information is abstracted and entered via a computer terminal into an on-line data collection system that edits data for consistency upon entry. The main file update system modifies clinical history files with new information and continues to edit for data consistency. Data concerned with epidemiology, therapy, histopathology, and survival as well as data about estrogen receptor status have been stored on over 500 patients. Personnel prepared extensive tabulations of these data for a meeting of the Breast Cancer Treatment Committee in May 1977.

Significance for Cancer Research (NCP Objective 6 Approach 5)

Project Officer: Mary E. Sears, M.D., Donald Corle, M.S.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $65,800

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Dr. Joseph Post
Name/Address: Goldwater Memorial Hospital
Performing Organization: New York University
New York, NY

Contract Number: NO1-CB-33903
Starting Date: 6/28/73 Expiration Date: 6/27/77

Goal: To study cytokinetics of human breast cancer.

Approach: I. In vivo: (a) ^{3}HTdR is given i.v. as a pulse label 7.5-10 mCi/pt. and autoradiographs of serial biopsies made to develop a labeled mitosis curve and to record grain count decay; (b) ^{3}HTdR is given as continuous label, 5 mCi/24 hr over several days and lesions are biopsied serially. These techniques permit estimation of population engaged in DNA synthesis, cell cycle parameters, and the source of new cells.

II. In vitro: (a) Enzymatically dissociated cells from biopsies and primary surgical specimens are studied by pulse and continuous labeling, along with double-labeling (^{3}H and ^{14}CTdR) for S times to define tumor cell proliferation. (b) Parallel kinetic studies are made on cultured cells as in IIa. Chromosome estimates are made on colcemid collected mitoses. Viability of cells in vitro is established by trypan blue exclusion, growth in culture, and ^{3}H uridine incorporation.

Progress: I. In vivo data (8 pts.) show small proliferating pools (<10%); PLM curve reveals T_{G2} + M/2 of ~4 hr and T_s of ~24 hr with no second wave in samples up to 139 hr; a wide range of intermitotic times, many cells spending long intervals in G_1 and in G_2. The PLM curve is composed chiefly of "fast replicators" (1,2). These data are confirmed by interphase grain count decay pattern (3).

II. Log phase cultured cells (from 13 pts.) have larger proliferating pools and faster replication than cells in vivo. Estimates of chromosome numbers ranged from pseudo-diploid to 120.

III. In 65 patients, collagenase-dissociated cells labeled in vitro had labeling indices and T_s similar to cells labeled in vivo. The validity of this method has been established by parallel studies in vivo and in vitro in 5 patients. Cytokinetic data provide a basis for planning and monitoring drug therapy and are being correlated with the clinical courses.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: New Techniques in Cell Kinetics of Breast Cancer

Principal Investigator: Dr. Robert M. Zucker
Name/Address Papanicolaou Cancer Research Institute
Performing Organization: Miami, FL

Contract Number: NO1-CB-33861
Starting Date: 6/27/73 Expiration Date: 2/26/77

Goal: Develop an approach to dissociate human mammary tumors into single cells to study cell kinetics and the cellular effects of chemotherapy.

Approach: A combination of mechanical and enzymatic techniques will be used to dissociate mammary tumor into single cells. The morphological architecture of the tumors will be correlated to the mechanical and enzymatic approach used. The dissociated cells will be characterized by biochemical and biophysical parameters which include electronic volume analysis, microfluorometry, ^{3}H-thymidine labeling indices, and fluorescein diacetate staining viability.

The in vivo effects of chemotherapy on the transplantable 13762 rat mammary papillary adenocarcinoma will be evaluated to obtain a predictive chemotherapy system. The evaluation will include standard growth measurements combined with biophysical cell separation, morphology, and cell kinetics.

Progress: The procedures to dissociate rat and mouse mammary tumors were optimized using a combination of enzymatic and mechanical procedures based on the morphological appearance of the tumor. The small tumor pieces were incubated at 37° in Ca^{++} free 199 medium containing collagenase, trypsin, DNA'ase, antibodies, and bovine serum albumin. These procedures used on animals were adapted to the dissociation of human tumors. Modification in the approach included a preincubation step of the small tumor pieces with the enzyme mixture at 4°C, the addition of Ca^{++}, and the removal of trypsin. The single cells obtained by this technique were analyzed by fluorescent flow analysis and ^{3}H-thymidine labeling for cell kinetic parameters. The cells were also analyzed by microscopic morphological parameters and fluorescein acetate viability.

Combination chemotherapy on 13762 tumors proved to be more effective than the single agent drugs. The effects of the chemotherapy could be monitored using cell separation procedures of velocity sedimentation and density gradients. However, the use of cell separation procedures was not effective in determining cell kinetic parameters on dissociated single cells from heterogeneous solid tumors.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/31/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Suppression of Endocrine Function by Systemic Agents for Breast Cancer Therapy

Principal Investigator: Dr. Richard J. Santen
Name/Address Pennsylvania State University
Performing Organization: Milton S. Hershey Medical Center
Hershey, PA

Contract Number: NO1-CB-53851
Starting Date: 5/15/75 Expiration Date: 5/14/78

Goal: To produce suppression of adrenal function with aminoglutethimide and to compare the effects upon human breast cancer with those of adrenalectomy.

Approach: Female patients with inoperable, recurrent, or metastatic breast cancer are admitted to this study which is expected to establish the optimal technique for aminoglutethimide (AG) blockage of adrenal steroid synthesis and glucocorticoid suppression of the reflex rise in ACTH. The effects of AG on extra-adrenal estrogen production, ovarian function, and the hypothalamic and pituitary control of prolactin secretion are also being studied. The differential actions of the D-L racemic and the D form of AG will be established. Finally, the effects on human breast cancer of endocrine suppression by this noninvasive technique will be compared with those of surgical ablation in a randomized trial.

Progress: Dose response studies suggest that aminoglutethimide inhibits estrogen synthesis at two separate sites of action, the adrenal itself and extra-adrenal peripheral tissues. The extra-adrenal actions of AG are important since plasma estrone and estradiol levels reach maximum suppression before total adrenal blockade is achieved. Based upon sequential studies during various regimens, a fixed dosage combination of 1000 mg of AG and 40 mg of hydrocortisone daily was chosen as standard therapy. This regimen allows uniform suppression of estrogen levels. To simplify patient monitoring, the use of plasma dehydroandrosterone-sulfate (DHA-S) was validated as a means of quantitating adrenal steroidogenesis. DHA-S levels are 20,000-fold higher than those of estradiol and more easily measured. The effects of AG on prolactin secretion and ovarian function were documented. Thirty-eight percent of 50 evaluable patients with metastatic breast carcinoma experienced objective tumor regression in response to the AG-glucocorticoid regimen.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., E. Brad Thompson, M.D.
Program: Breast Cancer Treatment Site Visit Date: 3/7/75
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $225,000

CONTRACT RESEARCH SUMMARY

Title: Investigation of Steroid Sulfation and Estrogen Binding in Human Breast Cancer

Principal Investigator: Dr. Thomas L. Dao
Name/Address Roswell Park Memorial Institute
Performing Organization: Buffalo, NY

Contract Number: NO1-CB-43900
Starting Date: 2/1/74 Expiration Date: 1/31/78

Goal: To determine the relationship of steroid sulfotransferase activity to estrogen receptor protein levels, to pathologic staging, and to risk of relapse.

Approach: Steroid sulfating enzyme activity and estrogen receptor (ER) levels have been assayed on breast tissue obtained from approximately 300 patients in three institutions. The evidence will be evaluated for or against correlation of the steroid sulfotransferase activity with risk for tumor metastasizing potential and with histopathological parameters. The predictive potentials of ER and sulfation activity will be compared.

Progress: A total of 266 patients from three cooperating institutions have been entered into the study. Based on an analysis of 145 patients, the data suggest that recurrence is related to a low level of DHEAS. Tumors generally synthesize much less E_2S, suggesting that the determining factor in the ratio of DHEAS and E_2S resides in the activity of DHEA sulfotransferases. The data strongly suggest that as tumor size and number of lymph node metastases increase, the tumor makes more DHEAS, but not more E_2S. In these cases, the more DHEAS the tumors make, the better the prognosis seems to be. Data to date do not seem to show a relationship between DHEAS and E_2S synthesis and the level of ERP. Longer follow-up is needed to confirm or refute the prognostic significance of steroid sulfating activity.

Significance for Cancer Research (NCP Objective 6 Approach 6,4)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $8,800

CONTRACT RESEARCH SUMMARY

Title: Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: Dr. Arthur H. Rosoff
Name/Address
Performing Organization: Rush-Presbyterian-St. Luke's Medical Center
Chicago, IL

Contract Number: NO1-CB-43991
Starting Date: 6/17/74 Expiration Date: 6/16/78

Goal: To evaluate the relative merits of early versus delayed combination chemotherapy in patients with metastatic breast cancer known to have responded to surgical oophorectomy.

Approach: Premenopausal patients with metastatic progressing breast cancer are evaluated 12 weeks following surgical castration. Those patients who are showing no change or tumor regression (partial or complete response) are randomly assigned to the maintenance phase of either (1) observation or (2) cyclic treatment courses of cyclophosphamide, 5-fluorouracil, and methotrexate (CMF).

When relapse is documented in patients assigned to observation, CMF treatment will be started. The results of these studies are expected to compare regression rate, time to progression, and survival in these two groups of patients.

Progress: About 100 patients have entered the oophorectomy phase of the study, but only 30 have been randomized to the maintenance phase. Because of the slow accrual, entry to the study has been terminated. The current patients will be followed and evaluated.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Occult Breast Cancer Metastases in Axillary Lymph Nodes

Principal Investigator: Edward J. Wilkinson, M.D.
Name/Address The Medical College of Wisconsin
Performing Organization: Milwaukee, Wisconsin

Contract Number: NO1-CB-74110
Starting Date: 7/1/77 Expiration Date: 6/30/78

Goal: To determine if the presence of occult lymph node metastases in axillary lymph nodes previously diagnosed as negative correlates with clinical recurrence of breast cancer.

Approach: The investigators will comprehensively restudy the lymph nodes removed from approximately 500 patients at the time of mastectomy for primary breast cancer. In all cases the initial routine histological examination of the nodes demonstrated no evidence of metastases. A pilot study on a subset will utilize serial sectioning of node material and the best spacing between sequential subsets of these slides can be determined by re-examination of the subsets. Five to ten year followup information will be available on all patients. The study will consider the following factors in relation to their risk for clinical recurrence: primary tumor type, grade and volume; node number removed, number positive and positive levels; metastatic volume; pretreatment and postreatment records; age; and survival information.

Progress: New contract

Significance for Cancer Research (NCP Objective 5B Approach 6)

Project Officer: Mary E. Sears, M.D., Donald Henson, M.D.
Program: Breast Cancer Treatment Site Visit Date: 3/1/77
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $108,500

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II Carcinoma of the Breast

Principal Investigator: Dr. Frank C. Sparks
Name/Address University of California
Performing Organization: Los Angeles, CA

Contract Number: NO1-CB-43917
Starting Date: 6/17/74 Expiration Date: 6/16/78

Goal: To evaluate the effectiveness of adding chemotherapy or chemoimmunotherapy stimulation to mastectomy for patients with Stage II carcinoma of the breast.

Approach: Patients with any number of positive axillary lymph nodes are eligible to enter this study, which randomly assigns one of the following treatment schedules: (1) cyclophosphamide, methotrexate, and 5-fluorouracil (CMF); (2) CMF plus BCG; or (3) CMF plus BCG plus tumor cell vaccine. The tumor cell vaccine consists of irradiated allogeneic breast carcinoma cells grown in tissue culture. CMF is given in 12 cycles over 64 weeks. Immunotherapy is given for 114 weeks. Treatment failure consists of either metastases or development of a new carcinoma in the contralateral breast. The patients undergo evaluation of immunologic parameters before treatment begins and at intervals throughout the study.

Progress: More than 100 women have been entered into the study. Metastases have been detected in six patients and new carcinomas in the contralateral breast have been diagnosed in two others. About 80% of the patients have had serial testing of immunocompetence by measuring delayed hypersensitivity to DNCB and to PPD. The increase in reactivity to repeated challenges to DNCB was less in breast cancer patients receiving CMF and BCG than in a group of melanoma patients receiving only BCG.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 10/10/75
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $223,000

CONTRACT RESEARCH SUMMARY

Title: Prediction of Hormone Dependency in Human Breast Cancer

Principal Investigator: Dr. Elwood V. Jensen
Name/Address University of Chicago
Performing Organization: Chicago, IL

Contract Number: NO1-CB-43969
Starting Date: 6/16/66 Expiration Date: 6/15/78

Goal: To develop techniques which will increase the predictability of response of human breast cancers to endocrine therapy.

Approach: Since 1966, Dr. Jensen has been studying estrogen receptors (ER) in human breast cancer tissues and the relationship between ER content and response to endocrine manipulative treatments. Because it now appears that patients whose breast cancers contain small or negligible amounts of ER have little if any chance of responding to endocrine therapy, whereas most but not all patients with tumors containing substantial amounts of ER receive benefit from hormonal manipulation, attention is directed toward three principal objectives: (1) understanding of why some receptor-containing breast cancers do not respond, (2) development of a simple and specific radioimmunoassay for ER in breast cancers, and (3) evaluation of the ability of an ER assay on the primary tumors, carried out at the time of mastectomy, to predict subsequent response to endocrine therapy.

Progress: Of more than 1,200 patients whose primary and/or metastatic breast cancers have been analyzed, approximately 70% can be classed as ER-poor and 30% as ER-rich. Of the treated patients with ER-rich tumors, 65% showed objective response as compared to very few of those with ER-poor tumors. Studies with animal models suggest that the sensitivity of the RNA polymerase system in isolated tumor nuclei to stimulation by estrogen-receptor complex may be a more definitive indicator of hormone dependence than is ER content. Immunoglobulin from rabbits immunized with highly purified ER from calf uterine nuclei was found to interact with estrogen-receptor complex from calf uterus; cross-reactivity with human breast cancer ER is being determined. Fifteen women, whose primary cancers were analyzed at the time of mastectomy, underwent endocrine therapy for recurrence four months to 5 years later; 3 of 4 patients with ER-rich tumors showed objective remissions, whereas 10 of 11 patients with ER-poor primary cancers failed to respond.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Mary E. Sears, M.D., Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 10/23/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Endocrine Therapy Plus Chemotherapy in Patients with Breast Cancer

Principal Investigator: Dr. David T. Kiang
Name/Address University of Minnesota School of Medicine
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-64000
Starting Date: 6/1/76 Expiration Date: 5/31/78

Goal: To study the effect on metastatic breast cancer of combining chemotherapy with estrogen therapy and to assess the value of estrogen receptor assays for treatment selection.

Approach: Patients eligible to enter this study program will be women who are showing the first evidence of metastatic breast cancer. Estrogen receptor (ER) assay results will determine the pair of treatment regimens to be randomly assigned after stratification according to disease-free interval and disease-dominant site. The patients with ER-negative cancer will receive (1) diethylstilbestrol (DES) plus cytoxan and 5-fluorouracil (CF) or (2) CF alone. Those with ER-positive cancers will receive either (1) DES and CF in combination or (2) DES alone followed by CF with evidence of DES treatment failure or of tumor progression after initial regression. The patient on whom ER assays cannot be obtained will have the same treatment assignments as those with ER-positive tumors.

The incidence and duration of remissions and survival data will be analyzed to determine (1) the effect of combining standard hormonal therapy and chemotherapy and (2) the value of ER assay results in predicting the clinical response to therapy.

Progress: About 50 patients have been admitted to the study. The overall remission rate is above 50%. It is too early to observe any differences among the treatments concerning the incidence or duration of disease remission.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Marc Lippman, M.D.
Program: Breast Cancer Treatment Site Visit Date: 2/11/76
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $90,200

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: John M. Yuhas, Ph.D
Name/Address University of New Mexico School of Medicine
Performing Organization: Albuquerque, New Mexico

Contract Number: NO1-CB-74203
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: The project will first explore the feasibility of growing six or more lines of rat mammary tumors in vitro as multicellular tumor spheroids (MTS). The characteristics of the in vivo tumors will be compared with those of the corresponding MTS. These characteristics will include transplantability into syngeneic rats or anergic mice, morphology, growth rate and growth fraction, cell cycle parameters, karyotype, and hormone receptors. If MTS are successfully developed, their responses to single drug doses will be studied and compared with in vivo responses. The in vitro MTS will be exposed to the test drugs in conditions which simulate the in vivo conditions. When metabolic activation of drug is required, sera from drug-treated animals will be tested. The test drugs will be those which are commonly used in the treatment of human breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment Site Visit Date: 1/18/77
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $96,300

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Group (NSABP)

Principal Investigator: Dr. Bernard Fisher
Name/Address University of Pittsburgh
Performing Organization: Pittsburgh, PA

Contract Number: NO1-CB-23876
Starting Date: 6/30/72 Expiration Date: 6/29/78

Goal: To determine the relative effectiveness of various local, regional and systemic treatments for clinical and pathological Stage I and II breast cancers.

Approach: Prospective, randomized clinical trials compare (a) the relative efficacy of radical mastectomy and total mastectomy with and without radiation in clinical Stages I and II breast cancer, and (b) total mastectomy with segmental mastectomy with and without radiation of the breast. Trials of systemic therapy evaluate (c) L-PAM vs. placebo, (d) L-PAM vs. L-PAM + 5-FU, and (e) L-PAM + 5-FU vs. L-PAM + 5-FU + MTX. Intermittent dosage schedules are employed for two years or until evidence of treatment failure. Additional trials employing (f) tamoxifen and (g) C. parvum were recently begun. A protocol to evaluate therapy in Stage III breast cancer is being revised for consideration and implementation. Similarly, consideration is being given to a protocol for histological Stage I breast cancer patients.

Progress: Patient entry has been completed in trials (a), (c), and (d) (see above) and they are being followed. In almost 1,700 patients who had undergone either radical, total or total mastectomy plus radiation therapy (Protocol No. 4), no significant differences have occurred in treatment failure or survival rates. The mean follow-up time is more than 36 months. In Protocol No. 5 (L-PAM vs. placebo), 400 patients have been followed for a minimum of 30 months. L-PAM treated patients aged $\leq$ 49 show fewer treatment failures than do those untreated. No significant benefit in patients aged $\geq$ 50 exists about 15 months postmastectomy. There are 700 patients in Protocol No. B-07 (L-PAM vs. L-PAM + 5-FU). Toxicity following the two drugs is not much greater than following L-PAM alone. Follow-up time is too short in that protocol and in Protocol No. B-08 (L-PAM + 5-FU vs. PMF) to present meaningful results. Patient accrual in the segmental mastectomy protocol is proceeding slowly. Extensive evaluation of the pathologic material submitted on patients in Protocol No. 4 has been carried out and has been published in a syllabus (Cancer 36:1-85, 1975). Information concerning estrogen receptors in these patients is being evaluated as are hormonal levels in blood of patients receiving chemotherapy.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment Site Visit Date: 12/14/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $499,500

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Russell Hilf, Ph.D.
Name/Address University of Rochester School of Medicine and Dentistry
Performing Organization: Rochester, New York

Contract Number: NO1-CB-74204
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: This project will perform assays for a battery of enzymes in primary and metastatic breast cancer specimens and build mathematical models of the biochemical profile. The enzymes include lactate dehydrogenase, pyruvate kinase, glucosephosphate isomerase, and isocitrate dehydrogenase. Hormone receptor assays and histological examinations will also be performed. Data concerning the hosts' biological and oncological histories will be aquired, evaluated and stored. Analyses of the combined data will test the predictiveness of the mathematical model constructed from the biochemical profile.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment Site Visit Date: 1/25/77
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds: $72,600

CONTRACT RESEARCH SUMMARY

Title: Methods to Predict Chemotherapy Sensitivity

Principal Investigator: Robert B. Livingston, M.D.
Name/Address University of Texas Health Science Center
Performing Organization: San Antonio, Tesas

Contract Number: NO1-CB-74205
Starting Date: 7/15/77 Expiration Date: 7/14/78

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: In vitro experiments will determine the effects of drugs on the thymidine labeling index of the rat mammary tumor 13762. The drugs will be directly added in pharmacologic concentrations or indirectly added via the serum of drug-treated animals. The in vitro effects will be compared with the in vivo effects on tumor size and on host survival. Cyclophosphamide, fluorouracil, methotrexate, vincristine, phenylalanine mustard, and adriamycin will be the drugs used in the test systems individually and in various combinations. If positive correlation between the in vitro and in vivo effects is found, additional experiments will test the predictiveness of the in vitro effects for the development of metastases and with the survival of animals from which the advanced local tumors have been excised.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)

Project Officer: Mary E. Sears M.D. Site Visit Date: 1/17/77
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY 77 Funds $54,100

CONTRACT RESEARCH SUMMARY

Title: Estrophile Binding and Estrophile Proteins in Human Breast Cancer

Principal Investigator: Dr. Federico Welsch
Name/Address Worcester Foundation for Experimental Biology
Performing Organization: Shrewsbury, MA

Contract Number: NO1-CB-43867
Starting Date: 10/16/73 Expiration Date: 10/15/77

Goal: To determine whether the levels and properties of estrophilic proteins are related to the responsiveness of cancer to endocrine treatment.

Approach: This is a type I contract in that the contractor carries out determinations of estrophilic receptor protein (ER) levels on 650 breast cancer samples per year. These tissues are submitted by collaborating clinicians whose protocols have been approved by the project officers. The ER assays are performed by both the sucrose gradient and the charcoal methods and methodology is under constant monitoring. Histological examination by St. Vincent's Hospital is being carried out on each tissue submitted for ER assay. The contractor also performs steroid sulfurylation determinations on tumor samples of sufficient size.

Progress: The contractor has reached the capacity of 650 assays per year and applications have exceeded that number. The project officers are receiving the reports of the ER assay results from the laboratory and are also provided with clinical data by the collaborating physicians. The combined data are being analyzed to determine the degree of correlation between ER levels and tumor response to treatment and other clinical and biological parameters.

Significance for Cancer Research (NCP Objective 5B Approach 6)

Project Officer: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 6/7/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 77 Funds: $135,000

ANNUAL REPORT SUMMARY
CANCER DIAGNOSIS CONTRACT PROGRAM
July 1, 1976 to September 30, 1977

The Diagnosis Branch of the Division of Cancer Biology and Diagnosis, which has the major responsibility for the Diagnosis Program of the National Cancer Institute, has probably reached its peak of expansion during this present year. As previously noted this program is almost entirely operated by means of the contract mechanism; with the exception of several CREGs this remains the status at present. Again, the program is defined and the contracts awarded and monitored with the expert advice of five individual Advisory Committees - one for each of the specific areas of involvement: General Cancer Diagnosis, Diagnostic Radiology, Breast Cancer, Cytology Automation and Immunodiagnosis. The programs for the last three are presented in this Annual Report in separate sections; those for the first two are presented below.

GENERAL CANCER DIAGNOSIS STUDIES

Lung Cancer By the end of the present fiscal year, it is likely that all three institutions (Mayo Clinic, Johns Hopkins University Medical School and Memorial Hospital-Strang Clinic) will have entered their full quotas of screenees, including controls. All three institutions continue to identify early lesions - both by cytology and by radiology - in an effort to permit lesion removal and cure by a surgical procedure that will be less extensive than pneumonectomy. The University of Cincinnati Statistical Center continues to coordinate accumulation of data from the three cooperating clinical projects. A Manual of Procedures, that was compiled early in 1975 and for which there have been considerable requests, will be reviewed and brought up to date before the end of this calendar year. All three Institutions continue to do some special studies that will add to the total value of the project.

Pancreas Cancer The three cooperating institutions, Mayo Foundation, University of Chicago, and Memorial Hospital will have finished their patient input into this study which attempted to define diagnostic methods with a high probability of finding pancreatic cancer at surgical exploration. In the process they have collected the largest group of possible pancreatic cancer, the members of which have been examined by almost all known pancreatic cancer detection devices and have gone on to surgical exploration. They have thus been able to establish many negative findings as well as the positive finding that ultrasound (using gray scale), C-T scanning and retrograde pancreaticoduodenography together will come close to identifying all pancreatic cancers (small pancreatic cancers were not among those explored or seen). Final reporting based on the data collected at the University of Cincinnati Statistical Center will take place within the next year.

Colonic Cancer The American College of Radiology continues to investigate the relative values of twelve methods of bowel preparation prior to barium enema or colonoscopy. The University of Louisville continues to investigate the development of an immunologic rapid method for the detection of human blood in stool. By the end of this fiscal year, the University of Minnesota will have recruited its full quota of screenees in its investigation of a screening technique using a test for blood in the stool as a means of

detecting early bowel cancer and thereby possibly prolonging lives; this study includes a control group and two study groups, one screened annually and one screened biennially.

Biochemical Markers Roswell Park Memorial Institute will have completed its development of a potential prostatic cancer marker.

Hormonal Markers Harbor General Hospital is continuing its investigation of hormonal markers in lung cancer, colonic cancer and breast cancer.

Nuclear Magnetic Resonance The two remaining groups working in this area, Downstate Medical Center (SUNY) and Baylor University College of Medicine, will have completed their contractual efforts during this fiscal year. It is likely that an algorithm may be able to distinguish between tissue containing colonic cancer and normal tissue.

Multisite Screening continues to be investigated at the University of Tennessee.

Systems to improve x-ray imaging are being developed and tested at University of Alabama and University of Wisconsin.

Periodic screening for medullary thyroid carcinoma is being done in relatives of patients at Duke University to investigate possibilities of altering mortality from this disease.

Immunodiagnostic Methods of detection for ovarian cancer are being developed and tested at Tufts University College of Medicine and at Roswell Park Memorial Institute.

The Development of a 5'-Nucleotide Phosphodiesterase Serum Enzyme Test for human liver cancer is being done at the University of Pennsylvania.

DIAGNOSTIC RADIOLOGY

In this area of research, improvement of x-ray imaging has been a matter of major concern and one to which the Diagnostic Radiology Committee has devoted much time. For early cancer detection and for screening programs involving periodic recurrent x-rays of individuals, it is extremely important to achieve improved resolution with substantially lower levels of radiation.

Five institutions: Massachusetts General Hospital, Columbia Presbyterian Medical Center, the Mayo Clinic, George Washington University Medical School and the Cornell University Medical School, have projects to perform clinical evaluation of equipment for computer assisted tomography of the brain, a non-invasive technique believed to be of great promise in the field of neuro-radiologic diagnoses. This technique is expected to complement and in some instances replace present techniques used in diagnosis of intracranial lesions. Enrollment of patients is being completed during the summer of 1977; follow-up studies will continue for about two more years. Extensive results have been obtained in this collaborative study; a number of reports currently are being prepared for publication.

A project to investigate use of proton beams and heavy ion beams in tissue density measurement is in progress at the ERDA-Lawrence Berkeley Laboratory. Final evaluation of possible clinical usefulness of the imaging method can not yet be made.

In addition to the above six projects, all of which were begun in June 1974, seven additional projects were begun in June 1975. All seven were concerned with various aspects of development of new cancer diagnostic capabilities using computer assisted tomography (CAT). One contract, with Mayo Foundation concerned accumulation of a transaxial x-ray projection data base for use in testing algorithms; another with Research Foundation of SUNY supported development of algorithms for computerized transaxial x-ray reconstruction. Both of those contracts have been most productive. The Mayo contract which terminated in June 1977 has produced an extensive projection data bank which will be made available by NIH to other interested groups. The SUNY contract, which will be completed in January 1978, will provide new useful algorithms for x-ray reconstruction. Three contracts, with ERDA-Lawrence Berkeley Laboratory, University of Pennsylvania, and Massachusetts General Hospital, involved development of algorithms for computerized transaxial nuclide reconstructions. The last two were completed in June 1977 and final reports will be available soon; the one with ERDA-LBL will terminate in June 1978. All three are producing a variety of new and useful algorithms for nuclide reconstructions; these will be made available to interested groups. Two contracts, one with American Science and Engineering and one with Presbyterian Hospital (NY), are supporting development and evaluation of a CAT body scanner. The scanner was completed and installed by AS&E at Presbyterian Hospital in May 1977. It currently is being optimized and clinical evaluation will begin soon.

During June 1976 four new contracts related to Diagnostic Radiology were begun. One, with Bolt Beranek and Newman will produce a standard protocol for use in evaluating imaging techniques used for cancer diagnosis. The tentative standard protocol has now been developed and will be pilot-tested during summer and fall 1977. Following any necessary modifications the standard protocol will be used to evaluate results accumulated from two other collaborative studies in the Diagnosis Program.

Two other contracts, with University of Kentucky and University of Illinois, are supporting studies to produce tumor-specific gamma-labelled immunoglobulins to allow tumor detection by external means. The fourth contract, with University of Arizonia, supports development of radioisotopically labelled markers which will accumulate in small cancer lesions in mucosal surfaces and the development of suitable detectors to locate such accumulations on endoscopically approachable surfaces.

During summer 1977 new contracts will probably be negotiated for studies in three new areas: (1) development of large area solid state image receptors for x-ray images; (2) development of ultrasonic probes to be inserted through endoscopes for improved cancer diagnosis; and (3) development of new techniques for improved passage of colonoscopes to the cecum to promote improved early diagnosis of bowel cancer.

CONTRACT RESEARCH SUMMARY

Title: Development of a Computerized Transaxial X-ray Reconstruction System

Principal Investigator: Dr. Jay A. Stein
Name/Address
Performing Organization: American Science and Engineering, Inc.
Cambridge, MA

Contract Number: NO1-CB-53858
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To establish a new radiologic process that will contribute toward earlier and more accurate detection, identification, and localization of cancer pathology in the chest, abdomen, and other anatomical regions, with potential low risk to the patient.

Approach: This study involves two cooperative contracts (NO1-CB-53858 and NO1-CB-53974). In the initial year, equipment and displays will be developed and fabricated for computerized transaxial x-ray reconstruction for parts of the torso analogous to present equipment used for such studies of the head. At the same time, suitable algorithms for radiologic cross-section images of high spatial densitometric and temporal resolution will also be developed. In the ensuing two years, clinical trials of equipment will be pursued on suitable human subjects and results compared with conventional diagnostic radiological and clinical examinations taken on the same subjects. Instrumentation and algorithm modifications suggested by engineering evaluations and clinical experience will be incorporated as warranted and feasible. Independent appraisal of the radiographic results and diagnostic success will be made by a panel of experts selected by the National Cancer Institute.

Progress: The body scanner design configuration was completed during the first year and fabrication of the unit was nearly completed by the end of the first year. Installation of the body scanner unit at Presbyterian Hospital, for evaluation under Contract NO1-CB-53974, originally scheduled for summer 1976, was made during March and April 1977.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Dates: 5/5/76; 3/3/77
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $250,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Standard Protocol for Evaluation of Imaging Techniques in Cancer Diagnosis

Principal Investigator: Dr. John A. Swets
Name/Address Bolt Beranek and Newman
Performing Organization: Cambridge, MA

Contract Number: NO1-CB-64010
Starting Date: 6/10/76 Expiration Date: 6/9/78

Goal: To establish a new general protocol for comparison and evaluation of imaging techniques used in cancer diagnosis in order to contribute to the improved recognition of earlier cancer pathology.

Approach: The protocol will be developed to specify (1) the psychophysical procedures that provide unbiased measures of the diagnostic accuracy provided by each imaging technique, and (2) the data analyses that provide appropriate measures of the medical efficacy as well as the cost-benefit relationships associated with an imaging technique. To aid in the standard protocol development a pilot study will be made involving an exploratory analysis of data from two collaborative studies sponsored by NCI. The pilot study and the full protocol will be completed within the first year of the contract. During the second year the protocol will be used to accomplish complete analysis of the two sets of NCI imaging data.

Progress: Standard protocol has been developed and currently is being tested in pilot study. Because of unanticipated difficulty in obtaining necessary data required for pilot study from the two collaborative studies, the pilot study of the standard protocol has been delayed. Currently it appears that the pilot study will be completed in December 1977 instead of June 1977. Final revision of the standard protocol will be completed soon after the pilot study is completed. Use of the standard protocol for comparison and evaluation of the imaging techniques studied in the two collaborative studies will begin early in 1978.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/3/77
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $203,952

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Sadek K. Hilal
Name/Address Columbia-Presbyterian Medical Center
Performing Organization: New York, NY

Contract Number: NO1-CB-43910
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide scan, angiography and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of January 1977, 437 patients have been studied, including 323 Group I (145 Group 1A, 94 Group 1B, and 84 Group 1C), 42 Group II, and 72 Group III.

Patient enrollment will be completed in June 1977 and the only remaining work will be continuing patient follow-up for two additional years.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/18/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $43,419 (est.)

CONTRACT RESEARCH SUMMARY

Title: Fabrication and Evaluation of Computerized Transaxial X-ray Reconstruction System

Principal Investigator: Dr. Sadek K. Hilal
Name/Address Columbia-Presbyterian Medical Center
Performing Organization: New York, NY

Contract Number: NO1-CB-53974
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To establish a new radiologic process that will contribute toward earlier and more accurate detection, identification and localization of cancer pathology in the chest, abdomen, and other anatomical regions, with potential low risk to the patient.

Approach: This study involves two cooperative contracts (NO1-CB-53858 and NO1-CB-53974). In the initial year of the study, equipment and displays will be developed and fabricated for computerized transaxial x-ray reconstruction for parts of the torso analogous to present equipment used for such studies of the head. At the same time, suitable algorithms for radiologic cross-section images of high spatial densitometric and temporal resolution will also be developed. In the ensuing two-year endeavor, clinical trials of the equipment will be pursued on suitable human subjects and results compared with conventional diagnostic radiological and clinical examinations taken on the same subjects. Instrumentation and algorithm modifications suggested by engineering evaluations and clinical experience will be incorporated as warranted and feasible. Independent appraisal of the radiographic results and diagnostic success will be made by a panel of experts selected by the National Cancer Institute.

Progress: Algorithm evaluations have been carried out to test (1) detector fan of rays instead of a source fan; (2) tradeoffs in resolution between number of views and number of readings per view; (3) effect of polychromaticity on image quality; (4) production of lateral and frontal reconstructions; (5) filters, using patient data; (6) effect of decentering the body scanner. A protocol has been established for clinical evaluation of the body scanner; the protocol will be subdivided into body regions with separate physicians directly responsible for each region.

During winter 1976-1977, preparations were made for installation of the scanner when delivered by American Science and Engineering. Space renovation was completed in March 1977, the scanner was installed during March and April 1977, preliminary runs with the scanner began in May 1977 and clinical evaluations started soon therafter.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 5/5/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $172,674 (est.)

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. D. Gordon Potts
Name/Address: Cornell University Medical School
Performing Organization: New York, NY

Contract Number: NO1-CB-43980
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of January 1977, 590 subjects have been studied. This includes 510 patients in Group I, 40 patients in Group II, and 40 normal subjects. These patients are referred for study by the Department of Neurology or Neurosurgery at New York Hospital and by Memorial Hospital. The isotope scans, EMI scans, angiograms, pneumograms and skull films are performed using projections that can be readily compared. The magnification and degree of distortion of every view may be determined.

Giant histological brain sections are being prepared on patients coming to autopsy to show the entire brain in the planes of the EMI sections; 66 cases have been studied in this manner. This will permit an accurate comparison of the EMI views with the other studies.

Patient enrollment will be completed in June 1977 and the only remaining work will be continuing patient follow-up for two additional years.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/19/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $54,440 (est.)

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Dr. T. F. Budinger
Name/Address ERDA -- Lawrence Berkeley Laboratory
Performing Organization: Berkeley, CA

Contract Number: YO1-CB-50304
Starting Date: 6/30/75 Expiration Date: 6/29/78

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for CT nuclide reconstruction based on transverse section emission data from human subjects are in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit location of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. Algorithms will be developed for computerized reconstruction of clinical data produced by transaxial nuclide projection machines. In addition, techniques for algorithm evaluation and comparison will be developed and applied. Algorithms, written in a high level programming language such as FORTRAN, together with phantom, animal and human emission and transmission projection data and reconstructions will be recorded on nine track industry compatible magnetic tape and delivered to the NCI. The NCI shall make independent evaluations and disseminate this information to other investigators.

Progress: The program library and user's manual contain methods for quantitatively depicting three-dimensional distribution of radiopharmaceuticals in the body. Algorithms include iterative least squares methods (3), ART (2), SIRT (2), backprojection of filtered projections (multiple filters), and filtering backprojection (multiple filters). The library includes 4 methods of weighting backprojections, and 8 techniques of compensating for attenuation. Studies have been done on phantoms and patients using these algorithms and include quantitative evaluation of brain and liver tumors and change in tumor volume with therapy. During the second year of the study additional critical testing of the utility and effectiveness of the library was done by an independent user; more than 300 computer runs were made and certain suggestions made for modification and reorganization. Two schemes of hard-wiring reconstruction algorithms are being developed to provide widespread usefulness of those techniques found to be suitable from experience with the above accumulation of reconstruction strategies.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/31/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $90,300 (est.)

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: Dr. Cornelius A. Tobias
Name/Address ERDA -- Lawrence Berkeley Laboratory
Performing Organization: Berkeley, CA

Contract Number: YO1-CB-40302
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: To evaluate the diagnostic potential of accelerated proton and various heavy ion beams in the detection of tissue abnormalities poorly or not detectable with x-rays; to develop techniques for the use of the particle beam of choice as a low dose adjunct to x-ray diagnostics.

Approach: With suitable phantoms and in vivo and in vitro biological specimens, the resolution and density detection sensitivity of various accelerated charged particle beams (H, He, C, O, Ne) will be compared using the LBL Bevatron/Bevalac for $Z \geq 2$ beams and the Harvard cyclotron for proton beams. In addition, the importance of beam energy and suitability of various detectors (such as plastic nuclear track detectors and photographic film) will be evaluated for the optimum beam particle-energy-detector system. Human diagnostic studies in cancer detection will be conducted with the system of choice. Comparisons of particle radiography with other noninvasive diagnostic techniques and pathology (when possible) will be made. An initial patient mammographic study with a few select patients will be conducted to develop particle radiographic techniques. A larger sampling will follow to accumulate sufficient statistics for diagnostic evaluation of particle mammography. Concurrently, initial particle radiographic studies in the diagnosis of soft-tissue abnormalities of the brain and skeletal abnormalities will be made.

Progress: Studies to date, with phantoms and biological targets (including freshly excised mammary specimens), indicate that radiography with heavy ion beams (C, O, Ne) using a multi-layered stack of plastic foils for image registration affords substantially better lateral and depth resolution than light ion beams (proton, alpha) using a stack of photographic film. The heavy ion/plastics system produces superior depth resolution but inferior lateral resolution when compared with a conventional x-ray/film system. Techniques for low dose (0.3-0.5 rad) patient carbon mammography and extremity radiography have been developed and twelve patients radiographed with promising results. Tumor structures in the human breast which were invisible to x-rays have now been seen with heavy ions. Initial work in optical and computer synthesis of the images from a single multi-layered plastic stack into a single composite image has been done with encouraging results.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/23/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $245,000

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. David O. Davis
Name/Address George Washington University
Performing Organization: Washington, DC

Contract Number: NO1-CB-43981
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of January 1977, 441 patients have been studied; this number includes 311 in Group I; 66 in Group II; and 64 normals (Group III). In follow-up studies 162 patients have been queried with 144 responses. Patient enrollment will be completed in June 1977 and the only remaining work will be continuing patient follow-up for two additional years.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/14/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $43,288 (est.)

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Dr. John A. Correia
Name/Address: Massachusetts General Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-53913
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects appears particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location and intensity of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project, algorithms will be developed for computerized reconstruction of clinical data produced by novel transaxial nuclide projection machines. Novel radionuclide imaging systems and appropriate algorithms will be emphasized. These algorithms, written in a high level programming language such as FORTRAN, together with appropriate phantom, animal and human projection data and reconstructions, will be recorded on nine track industry compatible magnetic tape and delivered to the National Cancer Institute for independent review and evaluation and dissemination.

Progress: Analytical methods and data collection protocols developed during the initial six months of the study now are in use with the scintillation camera and the positron cameras developed by the research group. Phantom studies and simulations are being used to test influence of many factors on image reconstruction. Rotating tables have been constructed for both scintillation and positron studies with phantoms and with human subjects.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/29/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator:	Dr. Paul F. J. New
Name/Address	Massachusetts General Hospital
Performing Organization:	Boston, MA

Contract Number: NO1-CB-43983
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of February 1977, some 381 subjects have been studied, including 304 in Group I (75 Group IA; 75 Group IB; 154 Group IC), 47 in Group II, and 30 in Group III.

Patient enrollment will be completed in June 1977 and the only remaining work will be continuing patient follow-up for two additional years.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 2/13/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $53,593 (est.)

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Hillier L. Baker, Jr.
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-43982
Starting Date: 6/15/74 Expiration Date: 6/14/78

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scan, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the x-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: As of February 1977, a total of 520 patients have been included in the study: 333 Group I (190 Group IA, 82 Group IB, 61 Group IC), 87 Group II, and 100 Group III.

Patient enrollment will be completed in June 1977 and the only remaining work will be continuing patient follow-up for two additional years.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 1/16/75
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $25,530 (est.)

CONTRACT RESEARCH SUMMARY

Title: Data Base for Testing Algorithms for Computerized Transaxial Reconstruction

Principal Investigator: Dr. Richard A. Robb
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-53857
Starting Date: 6/30/75 Expiration Date: 9/30/77

Goal: To obtain information which can be used by radiologists to expedite establishment of new computerized evaluation procedures for improved x-ray detection of early cancer lesions.

Approach: A transaxial x-ray projection data base suitable for testing computerized reconstruction algorithms will be assembled. The data base will include five distinct classes of transaxial planar projection information: simulated data; data from phantoms; excised organs and body specimens; live animals and human subjects. Real projection data will be obtained using x-ray sources and detectors of established physical characteristics and various geometries under controlled experimental conditions. Simulated projection data will be mathematically and stochastically defined in accordance with explicit anatomical, biological, and radiological models of normal and pathological physiologic states and of the data acquisition process. Projection data will be digitized and recorded on industry compatible magnetic tape and delivered to the National Cancer Institute, along with appropriate documentation. The National Cancer Institute will furnish the data to other contractors for evaluation, improvement, and comparison of reconstruction algorithms.

Progress: All necessary test, simulation, and tape formatting programs were written during the first six months of the study and several types of data in each of the five proposed classes were entered into the data base file. During the remainder of the two-year contract period, experiments will continue on phantoms, animals, and human subjects to acquire necessary additional data described in the contract. In addition, a systems architecture for efficient storage and retrieval of the data base files will be developed to facilitate selective access to and utilization of the data base contents.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/30/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial X-ray Reconstruction

Principal Investigator: Dr. Gabor T. Herman
Name/Address Research Foundation, State University
Performing Organization: of New York
Albany, NY

Contract Number: NO1-CB-53860
Starting Date: 6/30/75 Expiration Date: 1/31/78

Goal: To improve detection and diagnosis of early cancer lesions by means of improved radiologic imaging techniques involving new mathematical algorithms for computerized transaxial x-ray reconstruction.

Approach: Algorithms will be developed for computerized three-dimensional reconstruction of x-ray absorption techniques from projection data collected via various sources and detector geometries. The reconstruction will minimize the anatomical and densitometric distortion and artifactual effects in regions of medical interest and decrease both scan time and radiation risk of the patient. Criteria also will be developed for evaluating efficacy of the algorithms. The algorithms will be written in a high level programming language such as FORTRAN and assembled in a library which takes advantage of input and output commonalities and is modular and expandable. The library of algorithms plus suitable mathematical and actual test data and results will be written on nine track industry compatible tape and delivered to the National Cancer Institute for distribution to other investigators in the field.

Progress: An algorithm has been devised, implemented and tested which can be used in a scanning device requiring less than 10 seconds scan time to detect lesions of 1 cm diameter or less in the chest or abdomen. Data base creation currently is in progress. A realistic detailed phantom of the human thorax has been produced along with simulated polychromatic x-ray projections for it. Subroutines have been written for most of the algorithms to be incorporated into the programming system and theoretical groundwork has been established for comparative evaluation of algorithms. Work also is progressing on the required display program.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/30/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Radioisotope Surface Markers and Detectors for Endoscopic Techniques

Principal Investigator: Dr. James M. Woolfenden
Name/Address University of Arizona Medical Center
Performing Organization: Tucson, AZ

Contract Number: NO1-CB-64012
Starting Date: 6/30/76 Expiration Date: 6/30/78

Goal: To establish a means for improving early detection of small cancer lesions on endoscopically approachable surfaces in order to improve life expectancy of the patient.

Approach: A system will be developed for endoscopic detection of small cancer lesions in mucosal surfaces by means of tumor-seeking radioisotopic markers. The system will include one or more appropriate tumor-seeking radioisotopically labeled markers and a detector system suitably matched to the emission characteristics of the markers to provide optimum localization of lesions.

During the first year the work will concentrate on development and testing of the tumor-seeking markers. During the second year development work on the tumor-seeking markers will continue and the testing of the markers in the animal models will be expanded. In addition, the work will be started to develop suitable detectors.

During the third year any necessary additional development or modification of the radiolabeled tumor-seeking agents will be made; development and optimization of the detector system and its incorporation into endoscopic equipment will continue; and clinical evaluation of the system using a previously agreed upon protocol will be conducted.

Progress: Preliminary work to develop tumor-seeking markers is under way.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 11/5/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0 (Annual effort: $153,838)

CONTRACT RESEARCH SUMMARY

Title: Development of Immunoglobulins Labeled with Gamma-emitting Radioisotopes

Principal Investigator: Dr. Wayne A. Tompkins
Name/Address University of Illinois
Performing Organization: College of Veterinary Medicine
Urbana, IL

Contract Number: NO1-CB-64073
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To detect and localize, within the region of the body, cancer tissue too small to be found with currently available methods.

Approach: Detection and localization of early and/or small cancer lesions will be improved by development of immunoglobulins which are relatively specific to selected human tumor tissue components and which are labeled with suitable gamma-emitters to provide external localization of the tumor site.

During the first year CEA and TSA (tumor-specific antigen) will be isolated from human tumors, purified, and injected into animals for antibody production. The resulting antibodies, after separation and purification, will be tested *in vitro* and in suitable *in vivo* animal models. Concurrently, development of antibody labeling techniques will be started and growth of intestinal carcinoma cells initiated in nude mice.

During the second year the CEA and TSA will be produced in larger amounts, labeled, and tested *in vivo* in animals.

Progress: Both primary and metastatic GI-associated tumor material is being collected; this includes paracentesis fluid, liver tissue and primary colon lesions. Isolation and purification of CEA from this material are proceeding and animals have been injected with some of that material to begin anti-CEA immunoglobulin production. ^{51}Cr cytotoxicity studies have been started. A solid phase ^{131}I-anti-CEA binding test has been developed for quantitating CEA on the surface of HCT-8R cells. Preliminary work has been done on extractions of TSA from colon tumor tissue.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Dates: 9/24/76; 3/30/77
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0 (Annual effort: $116,581)

CONTRACT RESEARCH SUMMARY

Title: Development of Immunoglobulins Labeled with Gamma-emitting-Radioisotopes

Principal Investigator: Dr. David M. Goldenberg
Name/Address University of Kentucky Research
Performing Organization: Foundation
Lexington, KY

Contract Number: NO1-CB-64011
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To detect and localize, within the region of the body, cancer tissue too small to be found with currently available methods.

Approach: Detection and localization of early and/or small cancer lesions will be improved by development of immunoglobulins which are relatively specific to selected human tumor tissue components and which are labeled with suitable gamma-emitters to provide external localization of the tumor site.

During the first year the tumor localizing efficiency of ^{131}I-labeled anti-CEA immunoglobulins will be evaluated in animal studies. After their suitability is established by the animal studies, the materials will be tested in human subjects. The clinical testing may begin before the end of the first year and will be done first with advanced incurable cancer patients, then proceed to those with curable cancer, and finally, if the initial studies are successful, done with those having questionable cancer. Other radiolocalization systems based upon non-CEA tumor antigens will also be studied concurrently with those described above. However, since these systems are in earlier stages of development, no clinical trials will be made on them before the third year of the study.

Progress: ^{131}I-labeled anti-CEA immunoglobulin has been purified and tested for purity and suitability for human use. First clinical testing of the material will be done during the summer of 1977.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit: 9/23/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0 (Annual effort: $79,980)

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Dr. Stanley Baum
Name/Address University of Pennsylvania
Performing Organization: Philadelphia, PA

Contract Number: NO1-CB-53859
Starting Date: 6/30/75 Expiration Date: 9/29/77

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for computerized transaxial nuclide reconstruction based on transverse section emission data from human subjects is in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging permits not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit precise estimates of location of radioactivity in small body volumes that would be lost or obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. In the present project, algorithms will be developed for emission circumferential aperture reconstruction tomography (ECART) from clinical projection data collected by a new transaxial coded ring aperture radionuclide scanner with high count efficiency.

Progress: Four different encoding mask systems for performing radionuclide emission circumferential aperture reconstruction tomography (ECART) in an axial plane have been modeled and their performance characteristics analyzed. The four mask systems include the pinhole camera, a noise-like rotating mask, a multiple gear-mask system and a time-modulated aperture system. The systems have been compared by evaluating their total counting efficiencies and their signal-to-noise limits. An optimal ECART has been found to detect many more photons per counting period than detected by a conventional collimated detector system; however, for distributed radiation sources both systems have approximately equal signal-to-noise ratios. ECART also has shown greater flexibility than collimated detector systems for optimally reconstructing objects of different sizes.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: R.Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 3/31/76
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator: Dr. Arthur J. Present
Name/Address American College of Radiology
Performing Organization: Chicago, IL

Contract Number: NO1-CB-43974
Starting Date: 6/15/74 Expiration Date: 6/14/77

Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time, excessive dietary restrictions, excessive patient discomfort or inconvenience.

Approach: Under administrative supervision of the American College of Radiology, the comparative efficiency of 12 selected colon cleansing regimens are being studied by 6 institutions. A total of 3,600 patients are being examined; all regimens will be employed by each institution on a randomized basis. Standard forms for collection of pertinent clinical information and for evaluation of efficiency of each regimen have been prepared. A panel of readers will independently examine x-ray film taken on the patients and evaluate the relative clarity of the bowel. A method for rerandomized distribution and evaluation has been requested per the last site visit. This will continue to encompass methods for cross evaluation. Statistical analyses will be performed by the Department of Biomathematics at M.D. Anderson Hospital.

Progress: The project is continuing with participation of 6 centers. Each center is responsible for preparing groups of 25 patients by each of 12 specified protocols. Projects range presently from a minimum of three quarters of completed protocols at one institution to all protocols completed at two institutions. Based on current data evaluation being conducted on protocols indicates two of the 12 specified protocols are superior to the others. Evaluation is also being conducted on film readers by way of comparison of variances between readers and frequency of rating patterns. The number of readers has been increased and an improved method of randomization of film reading will be instituted.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officers: Louis P. Greenberg and R.Q. Blackwell, Ph.D.
Program: Diagnosis Site Visit Date: 4/16/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0 (Extended Without Funds)

CONTRACT RESEARCH SUMMARY

Title: Determination of Optimal Frequency of Screening Strategies

Principal Investigator: Dr. Arthur Albert
Name/Address
Performing Organization: Boston University
Boston, MA

Contract Number: NO1-CB-53863
Starting Date: 6/1/75 Expiration Date: 5/31/77

Goal: To develop a means whereby the optimum frequency of screening can be determined for early cancer for various anatomic sites in order to assure success in such screening at the least possible cost and hazard to the patient and still be effective in reducing death from cancer.

Approach: A team of clinicians, pathologists, statisticians, operations researchers and epidemiologists will carry out extensive investigation and documentation of the level of present knowledge, both empirical and theoretical on the following five factors: (1) the natural history or development of the disease, (2) the incidence of the disease in the target population; (3) the effectiveness (sensitivity and specificity) of the various available screening tests at each stage of the disease; (4) the effectiveness (on mortality) of intervention (treatment during each stage of the disease); and (5) the cost and hazards of the various screening tests. Disease models will be developed and validated for which screening data exist, or must be developed, such as colon, lung, bladder and prostatic cancer. Through parametric analysis these models will be used to suggest general ranges or minimum limits on the performance of screening tests if they are to be effective in early cancer detection and reducing cancer mortality.

Progress: Accomplishments during this 2-year contract include the following: (1) Development of a time-dynamic model: The joint frequency of age and natural history sojourn times represent a statistical picture of the disease natural history in the population. (2) Development of optimum individual screening policies: Exercising this model has allowed evaluation of the objective function for any specified policy or search for an optimal policy. (3) Development of an urn model for the natural history of cancer in a population at an instant of time: This model allows for calculation of temporal changes in prevalences, incidences and epidemiologic descriptors. (4) Development of estimation techniques for determining statistical properties of the disease natural history in a population: This allows for accurate determination of occurrence rates of non-observable events such as the age-specific incidence of preclinical disease.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 5/3/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $7,700

CONTRACT RESEARCH SUMMARY

Title: Screening for Medullary Carcinoma of the Thyroid Using Calcitonin Radioimmunoassay

Principal Investigator: Dr. Samuel A. Wells
Name/Address Duke University
Performing Organization: Durham, NC

Contract Number: NO1-CB-63994
Starting Date: 6/15/76 Expiration Date: 6/14/78

Goal: The goal of this project is to establish a periodic screening program of relatives of patients with medullary carcinoma of the thyroid gland (MCT).

Approach: A cohort of patients in which MCT is proven histologically will be established. The cohort will be sufficiently large to offer a statistically significant number of family members. Those family members will be screened for serum calcitonin levels by radioimmunoassay methods in order to find MCT during its early stage of development. A long-term follow-up program will be conducted on these families to determine the natural history of the disease and effectiveness of therapy.

Progress: Since inception of the contract 238 persons were screened and 23 new cases of medullary carcinoma of the thyroid gland (MCT) were diagnosed. Members of six kindreds are being evaluated and the study population is well defined. Approximately 80 patients who have been operated on in the past or who currently have active disease are followed. Comparability of radioimmunoassays have been established between the two laboratories and standard and test antisera continue to be interchanged at regular intervals.

Evaluation of provocative testing is almost completed and it appears that the combination of calcium gluconate (2 mg/kg/one minute) and pentagastrin (0.5 mcg/kg/five seconds) is the best provocative test for stimulating calcitonin release from MCT cells. Furthermore, the evaluation of the utility of selective venous catheterization combined with pentagastrin stimulation in early diagnosis and localization of metastatic tumor foci of MCT is nearing completion. Twenty-five patients have undergone surgery for MCT and six have had adrenalectomies for pheochromocytomas.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $111,000

CONTRACT RESEARCH SUMMARY

Title: Hormone Markers for the Detection and Diagnosis of Cancer

Principal Investigator: Dr. William D. Odell
Name/Address Harbor General Hospital
Performing Organization: Torrance, CA

Contract Number: NO1-CB-43903
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To develop hormone markers that might indicate the presence of small (early), treatable cancer.

Approach: (1) To measure ADH, vasotocin, oxytocin, α and β MSH, ACTH, α and β TSH, and α and β HCG to search for tumor elaboration of these peptides among cancer patients; (2) to develop new techniques for separating and identifying "prohormone" forms of ACTH, gastrin, and pancreozymin-cholecystokinin (PZ-CCK); (3) to search for the existence of such big forms of ADH, oxytocin, α and β MSH, luteinizing hormone, and α and β glycopeptides, (4) to develop peptide assays for fetal hormones related to vasopressin; and (5) to identify other nonhormonal peptides which might be used as cancer markers. The contractor hypothesized that "ectopic peptide elaboration is a universal concomitant of neoplasia." This hypothesis is based on a review of several hormonal syndromes produced by nonendocrine cancers and the fact that, where known, they are produced by peptides. Production of nonbiologically active peptides is even more common than production of active peptides.

Progress: The elaboration of several peptides by several common cancers (lung, colon, breast, and prostate) has been investigated. In addition, smaller numbers of less common cancers have been studied. For each tumor type, the usefulness of six peptides (big- or pro-ACTH, β lipotropin, alpha chain of glycopeptides, beta chain, HCG, vasopressin-vasotocin, and a PTH-related peptide) with weak biological activity as tumor markers has been evaluated.

Results indicate that the hypothesis is valid. Over 95% of 87 tumor extracts contained greater amounts of these peptides than blood concentration. In a prospective blind study of patients with abnormal chest x-rays, 83% of those subjects shown to have lung cancers had elevations of one or more of these peptides. The following elevated levels were found in the blood plasma of lung cancer patients: ACTH-72%; BLPH-62%; α-chain, men-11%; α-chain, women-6%; β chain-7%; AVP/AVT-41%. Similar studies are nearly completed for colon cancer, and are in progress for breast cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/14/77
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $230,402

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer Control: Detection and Treatment

Principal Investigator: Dr. John K. Frost
Name/Address
Performing Organization: Johns Hopkins University
Baltimore, MD

Contract Number: NO1-CN-45037
Starting Date: 9/1/73 Expiration Date: 7/15/77

Goal: To determine the effect of the early detection of lung cancer by sputum cytology, followed by surgical removal of the tumor on long-term survival in the disease (Phase II of Hopkins Study).

Approach: The study population consists of males, 45 years or older, who smoke a pack or more of cigarettes a day. By random assignment from individuals responding to advertisement, it was planned to accumulate over several years two groups of over 5,000 each. The test group would receive sputum cytologic examination and chest x-ray every four months; the control group will have an annual chest x-ray. Subjects suspicious or positive for cancer would be carefully worked up, to include fiberoptic bronchoscopy; cancers would be removed surgically. All subjects will be followed for at least 5 years to determine actuarial survival. (See also NO1-CB-92172.)

Progress: Approximately 10,000 people have been screened to date in this project. A return rate of 3% was obtained from miscellaneous groups contacted by mailings; a return rate of 5% was obtained from the other subjects in industry and government. Screening was done at Johns Hopkins Hospital and Social Security office. Cancers are found to date in approximately 6 per 1,000 screenees (5.4/1,000 in the control X-group and 6.8/1,000 in the cytology C-group). For every 5 cases of lung cancer detected by chest x-ray there exist 2.06 additional cases of lung cancer detected only by cytology. Localization of these early lesions has been made by techniques of bronchoscopy and cytopathology. This project is carried on in conjunction with Mayo Clinic, Memorial Hospital, and University of Cincinnati in the NCI Cooperative Early Lung Cancer Group.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early cytologic detection, the best present hope for improving lung cancer mortality, requires rigid proof of its effectiveness.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $27,400 (Supplement to DCCR funding)

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer -- Early Detection, Localization, and Therapy

Principal Investigator: Dr. John K. Frost
Name/Address
Performing Organization: Johns Hopkins University
Baltimore, MD

Contract Number: NO1-CB-92172
Starting Date: 6/28/69 Expiration Date: 6/27/77

Goal: (1) Develop improved cytologic methods for sputum examination for the early detection of lung cancer; and (2) develop improved methods for the diagnostic localization of lung cancer (Phase I of Hopkins study).

Approach: The project follows groups of subjects who are at high risk of lung cancer: chemical and asbestos workers who are also cigarette smokers. They are tested by sputum cytology and x-ray studies. These studies are directed to the biologic significance of the morphology of mild, moderate and severe cell atypia by correlation with patient follow-up. Patients cytologically suspicious or positive for cancer are studied for better localization techniques, mainly with tantalum dust bronchography and fiberoptic bronchoscopy. Improved equipment is also under development. (See also NO1-CN-45037).

Progress: Clinical material was obtained from 2,165 cigarette smokers who are either asbestos or bis-CMME workers. Improved transbronchial television microscope was developed as well as new methods to diagnose cancer cytologically and histologically from areas of lung heretofore unaccessible. For x-ray negative lesion localization, controllable catheter tantalum bronchography and various instruments were developed for rigid and flexible fiberoptic bronchoscopy. Surgical techniques were developed for improved therapy of early lesions. Techniques evolved from this Phase I are used in Early Lung Cancer Cooperative Study. The project includes continuing investigation of biologic significance of cytologic high-suspect lesions; parameters for automatic recognition of lung cancer; development of transbronchoscope probes for mucosal tension, bronchial wall firmness, infrared sensing, thermorecovery, laser, electrocautery, and ultrasonic forceps; and development of clinical inhalation for tantalum bronchography.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early detection is the best present hope for improving the mortality from lung cancer.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0 (Extended Without Funds)

CONTRACT RESEARCH SUMMARY

Title: Lung Project for Detection and Localization of Early Lung Cancer

Principal Investigator: Dr. Robert S. Fontana
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-53886
Starting Date: 9/27/74 Expiration Date: 9/26/77

Goal: To improve methods of diagnosis of early lung cancer and to assess survival of patients with lung cancer detected by these methods.

Approach: A study population of 9,313 subjects was obtained from interviews of 11,000 non-volunteers. Subjects were Mayo Clinic outpatients who were men, 45 years old more, smoking one pack of cigarettes or more daily. None had a history or suspicion of respiratory cancer on entry into the Clinic; all had a life expectancy of at least 5 years, and all were judged capable of tolerating pulmonary resection. The 9,313 study subjects were first screened by means of chest x-rays, 3-day sputum cytology tests and lung health questionnaires. This screening yielded 86 unsuspected ("prevalence") lung cancers, 16 detected by cytology alone, 53 by x-ray alone and 17 by both tests. Radiographically "occult" cancers were localized fiberbronchoscopically. Subjects with a "negative" initial screen were randomized into a close surveillance group, rescreened every 4 months, and a standard surveillance group ("control") advised to undergo rescreening once a year. Potential lung cancer "risk-factors," including aryl hydrocarbon hydroxylase (AHH) are also being evaluated, as are hemato-porphyrin derivative (HpD) and cryotherapy as techniques for "mapping" and treating carcinoma-in-situ.

Progress: As of April 1, 1977, both the close surveillance group and the "control" group have been observed more than 10,000 man-years, and 94 new ("incidence") cases of cancer of the respiratory tract had been detected, 17 involving the upper airway and 77 the lungs. In the close surveillance group there were 53 new lung cancers (only 13 of which were detected cytologically) and 15 lung cancer deaths. Early survival data in this group are encouraging, but much longer observation is needed. In the control group there were 24 new lung cancers and 14 lung cancer deaths, with a tendency toward more traditional lung cancer survivorship.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 77 Funds: $1,050,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Usefulness of Carcinoembryonic Antigen in the Diagnosis of Bowel Cancer

Principal Investigator: Dr. Vay Liang W. Go
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-23854
Starting Date: 6/15/74 Expiration Date: 6/14/78

Goal: (a) Evaluate the CEA test in diagnosis of large bowel carcinoma; (b) continue evaluation of CEA as a biologic marker in patients undergoing chemotherapy, radiotherapy and immunotherapy of advanced gastrointestinal cancer; (c) measure CEA levels in blood and other specimens stored at NCI-Mayo Clinic Tumor Plasma Bank (CB-23879), Mayo Lung Cancer Project (NCI-CB-22000) and other contracts with the Immunodiagnosis Program of the Division of Cancer Biology and Diagnosis; (d) immunological quantification and chemical characterization of CEA-like material in secretions of the gastrointestinal tract; (e) continue to collect and provide colonic malignant tissue for different investigators in projects dealing with carcinoembryonic antigens.

Approach and Progress: (a) Results of serum CEA evaluation of 6,700 patients were previously reported. To date, follow-up information has been obtained on 531 normal subjects seen and followed over the past three years. None of the 15 normal subjects who had CEA > 5 ng/ml have developed detectable malignant neoplasms. Follow-up survival data of the patients who had colorectal cancer at the time of initial CEA determination have now been obtained. Among colorectal cancer patients with localized or with distant metastases involving the liver, those patients with an initial serum CEA < 5 ng/ml have a much better survival rate than patients with CEA > 5 ng/ml. (b) Serial determinations of serum CEA have been done on 612 patients with far advanced gastrointestinal malignancies who are currently being treated under various approved NCI protocols receiving chemotherapy, radiotherapy, and/or immunotherapy and 236 patients with colorectal cancer who were operated on with curative intent. Changes in CEA levels are currently being correlated with patient course, effect of cancer therapy, and relationship to other blood tests and measurable agents. (c) Immunochemical characterization of the CEA-like material from normal human colon washings shows it to be similar to tumor CEA.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 2/9/77
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $160,000

CONTRACT RESEARCH SUMMARY

Title: Detection of Pancreatic Cancer at Early or Small Stage Prior to Metastasis

Principal Investigator: Dr. Vay Liang W. Go
Name/Address Mayo Foundation
Performing Organization: Rochester, MN

Contract Number: NO1-CB-43895
Starting Date: 6/15/74 Expiration Date: 6/14/77

Goal: (a) To determine values of newer diagnostic tests in the detection of pancreatic cancer; (b) to determine whether surgical intervention of a localized pancreatic cancer significantly improves survival rate; and (c) to develop new procedures for pancreatic cancer diagnosis.

Approach: Three cooperating institutions will study, by a common protocol, patients who are suspicious of pancreatic cancer (PC) and warrant exploratory laparotomy. Tests to be done prior to surgery: CEA, alpha-fetoprotein and immunoglobulins, duodenal aspiration and study of pancreatic enzymes (PFT) and biliary secretion, ultrasonic abdominal study (U), fiberoptic duodenoscopy with retrograde pancreatocholangiography (RPGM), pancreatic scan with ^{75}Selenomethionine (S), selective celiac and/or superior mesenteric angiogram (A), and thermogram (T). At operation, portal blood will be obtained for additional studies. If pancreatic cancer is found at surgery, the patients will be evaluated at 3-month intervals for 3 years. If no cancer is found, patient will be evaluated at 6-month intervals for 3 years.

Progress: To date, 135 patients have been entered into the study. Analyses of the prospective comparison of current diagnostic tests on the first 70 patients surgically explored has now been completed. Pancreatic disease was detected in 90% of instances with pancreatic function tests and in 74% with ultrasound. Quantification of pancreatic CEA output did not differentiate all pancreatic cancer patients from other patients in any test condition. The pancreatic scan gave many false-positive results and thermography was insensitive. Arteriography, endoscopic retrograde pancreatography, and cytologic studies were 69%, 95%, and 23% accurate in diagnosis of pancreatic cancer. From these results, it is suggested that, when pancreatic cancer is suspected, abdominal ultrasound be given initially and if negative a pancreatic function test next. Positive results from either warrant endoscopic retrograde pancreatography for definitive diagnosis of pancreatic cancer. That sequence of tests in the present series identified 88% of patients without pancreatic disease and 89% with pancreatic cancer. Studies of various serum tumor markers were not helpful with the exception of CEA. The comparative value of computerized tomography to the above diagnostic tests are currently under evaluation.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/21/77
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $146,000

CONTRACT RESEARCH SUMMARY

Title: Detection of Pancreatic Cancer at Early or Small Stage Prior to Metastasis

Principal Investigator: Dr. Patrick J. Fitzgerald
Name/Address
Performing Organization: Memorial Hospital for Cancer and Allied Diseases
New York, NY

Contract Number: NO1-CB-43975
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To evaluate diagnostic methods and findings in patients undergoing abdominal exploration for supposed pancreatic cancer in order to establish a base that can be used for the detection of small pancreatic cancer before metastasis that may lead to an increase in the 5-year survival from 1% now prevalent to 13% or more.

Approach: Three cooperating institutions will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor-associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

Progress: In 31 months about 1,050 patients with abdominal complaints were seen by the Pancreas Study Group. Of these patients, 160 (15%) were suspected of having pancreatic cancer and 76 (64%) were found at operation to have it. Twenty-two patients (43%) had "curative" resections; 13 of these are living 2-20 months postoperatively. The operative mortality (death within 1 month) was 18%. In 43 other patients, at operation, 32 had non-pancreas cancer and 11 had noncancerous diseases. Computerized transaxial tomography was the most accurate diagnostic technique -- it diagnosed all 28 cases of proven pancreas cancer on whom it was tried but it gave 4 false positive diagnoses (2 cases of pancreatitis). Celiac arteriography diagnosed about 80% of cases with about a 20% false positive rate. Ultrasonography and selenium-75 scanning were less helpful. Carcinoma in situ was found in a small percentage of cases. Of about 15,000 asymptomatic patients screened at the Strang Clinic Institute, 15 were proven to have gastrointestinal cancer, but no case of cancer of the pancreas was found (about 2 cases were expected from incidence rates). Subsequently, 2 of the 15,000 patients were said to have died of pancreas cancer.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/21/77
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $186,560

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer -- Early Detection, Localization and Therapy

Principal Investigator: Dr. Myron R. Melamed
Name/Address
Performing Organization: Memorial Hospital for Cancer and Allied Diseases
New York, NY

Contract Number: NO1-CN-45007
Starting Date: 9/1/73 Expiration Date: 8/31/77

Goal: To evaluate sputum cytopathology as a supplement to annual chest x-rays in detecting pulmonary neoplasms at an "early stage" and to evaluate the efficacy of techniques for prompt localization of radiologically occult lung cancer (e.g., before progression to x-ray positive); in general, to evaluate the efficacy of such screening to reduce lung cancer mortality.

Approach: Over a 3-year period, 5,000 test subjects and 5,000 control subjects will be entered into this study with active screening for 5 years, all to be followed for at least an additional 5-year period, for sufficient years to be statistically significant to answer pertinent questions. This will be conducted according to a protocol developed in conjunction with the Johns Hopkins University and the Mayo Foundation.

Progress: The early lung cancer detection program in New York is now in its fourth year. The study is being conducted by Memorial Sloan-Kettering Cancer Center using facilities at the Strang Preventive Medicine Institute for interviews and examinations of the subjects involved. To date, over 9,300 men have been examined; it is anticipated that 10,000-12,000 will be entered into the program to be followed for at least five years. All are cigarette smokers over the age of 45 years. A comparison will be made between randomly selected subjects having annual chest x-rays only, and subjects having 4-monthly sputum cytology as well as annual chest x-rays. In a preliminary evaluation of the first 6,612 men, the initial examination revealed 15 lung cancers, Stages II and III, and eleven lung cancers, Stages 0 and I. Of 3,387 men who had x-rays alone, there were 3 early cancers detected (Stage I); of 3,225 men who had x-rays and cytology, there were 4 early cancers detected by x-rays (Stage I) and 4 detected by cytology (Stage 0). In addition, 3 larynx cancers were detected by cytology.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Lung Cancer Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 77 Funds: $587,256 (DCCR Funding)

CONTRACT RESEARCH SUMMARY

Title: Determination of Optimal Frequency of Screening Strategies

Principal Investigator: Dr. Michael J. Brennan
Name/Address Michigan Cancer Foundation
Performing Organization: Detroit, MI

Contract Number: NO1-CB-53907
Starting Date: 6/1/75 Expiration Date: 5/31/77

Goal: To develop a means whereby the optimal frequency of screening can be determined for early cancer of various anatomic sites in order to assure success in such screening at the least possible cost and hazard to the patient and still be effective in reducing death from cancer.

Approach: A team of clinicians, pathologists, statisticians, operations researchers and epidemiologists will carry out extensive investigation and documentation of the level of present knowledge, both empirical and theoretical on the following five factors: (1) the natural history of development of the disease; (2) the incidence of the disease in the target population; (3) the effectiveness (sensitivity and specificity) of the various available screening tests at each stage of the disease; (4) the impact (on mortality) of intervention (treatment during each stage of the disease); and (5) the cost and hazards of the various screening tests. Disease models will be developed and assessed for sites on which screening data already exist, such as colon, breast and cervical cancer. Lung, bladder and prostatic cancer models will also be undertaken to help define the requisite characteristics of screening techniques for these diseases. Through appropriate statistical and mathematical methods these models will be used to suggest general ranges or minimum limits on the performance of screening tests if they are to be effective in early cancer detection and reducing cancer mortality.

Progress: The models of breast, cervical, and colorectal cancer have been modified and extended. This work has been based on the feedback received in the preliminary analysis phase of the project completed in the first year. A Semi-Markov model has been developed that incorporates the underlying principles of the site-specific analyses. A decision theoretic framework has been used to analyze the number of tests to be given and the decision rules to be followed at a given screening examination. Finally, literature on lung and prostate cancer has been reviewed as a basis for formulating the analyses for these sites.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 5/3/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $159,720

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Malaya Bhattacharya
Name/Address Roswell Park Memorial Institute
Performing Organization: Buffalo, NY

Contract Number: NO1-CB-64013
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: The goal of this project is the development of sensitive immunoassay procedures for early diagnosis of ovarian cancers and serial monitoring of patients undergoing therapy for ovarian cancers.

Approach: The investigation will be conducted on the following topics: (1) preparation of antisera (anticystadenocarcinoma sera); (2) sources of ovarian cystadenocarcinoma-associated antigens (OCAA); (3) purification of OCAA by conventional methods, immunoadsorbent columns, affinity columns of plant lectins; (4) determination of purity of OCAA; (5) characterization of OCAA; (6) determination of the frequency of occurrence of OCAA in other cancer and non-cancer tissues; (7) development of a radioimmunoassay for detection of OCAA; (8) patient selection, availability and follow-up; (9) characterization of immune response to ovarian cancers with emphasis on cystadenocarcinoma; and (10) problems and limitations of the proposed projects.

Progress: Evidence has been found for the presence of at least three different tumor-associated antigens or antigenic determinants in human cystadenocarcinomas of the ovary. Solid ovarian tumors, tumor cells in ascites fluids and cyst fluids from ovarian cancer patients were used as sources for these antigens. Antisera were raised in rabbits against cystadenocarcinoma extracts. After proper adsorptions the antisera produced were used to perform the assays for tumor-associated antigenic reactivity. For quantitative isolation of these antigens repeated extractions separately or in combinations with 3M KCl, 6M urea and normal saline were performed; 3 time extraction with saline gave the best results. Ammonium sulfate fractionation of the saline extracts gave about six- to ten-fold purification. Further fractionation of the saline extracts gave about six- to ten-fold purification. Further fractionation by gel filtration and ion-exchange chromatography has yielded about 120-fold purification of the antigens. Antisera now are being produced in rabbits against these preparations. Further purification of the antigens utilizing immunoadsorbent columns and affinity columns are under way.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 12/14/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $55,453

CONTRACT RESEARCH SUMMARY

Title: Biochemical Markers or Enzyme Changes That May Presage the Presence of Cancer

Principal Investigator: Dr. Alexander Brownie
Name/Address State University of New York
Performing Organization: Buffalo, NY

Contract Number: NO1-CB-43977
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To develop and establish a highly sensitive test for a specifically prostatic form of serum acid phosphatase as a marker for early prostatic cancer.

Approach: It is intended to prepare in bulk the isoenzymes of human prostatic acid phosphatase, by methods previously proven in this laboratory. Both the water-extracted types, which are believed to be secreted in the prostatic fluid, and the detergent-extracted type (obtained by a Tween method) will be examined separately. Each will be used as an antigen to obtain antibody and other reagents for a radioimmunoassay method, for its specific detection at very low levels in sera. Each will also be studied for any specific binding or inhibitory property that can characterize it at low levels in the presence of other phosphatases.

Progress: The acid phosphatase from cancerous human prostatic tissue was purified and a rabbit antiserum to this purified enzyme was prepared. The antiserum is specific for the prostate enzyme; it did not cross react with acid phosphatases from other human tissues, such as kidney, liver, lung, spleen, red blood cells, etc. With this purified enzyme and its antiserum, a sensitive and specific immunochemical method has been developed, which detects as low as 0.2-0.4 IU or 10-20 ng/ml of prostatic acid phosphatase. With this method it was possible to detect 20% of stage B, 50% of stage C and 80% of stage D prostate cancers. The isoenzymes of human prostatic acid phosphatase were studied by an isoelectric focusing technique. Purified acid phosphatase from malignant prostatic tissues contained eight distinct isoenzymes with pI 4.7-5.3. The sera from patients with prostatic cancer were shown to have similar acid phosphatase isoenzymes. Serum RNase activities were also found to be significantly increased in patients with prostatic cancer when compared with those in normal healthy individuals.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/17/75
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $79,724

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Leo Stolbach
Name/Address
Performing Organization: Tufts University School of Medicine
Boston, MA

Contract Number: NO1-CB-64028
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: The goal of this project is to develop a screening test for the early diagnosis of ovarian carcinoma in asymptomatic women.

Approach: Malignant effusion of patients with ovarian carcinoma will be processed in order to (1) isolate and purify tumor-specific antibodies and/or antibody-antigen complexes from effusion fluids; (2) dissociate the antibody-antigen complexes and separate the tumor-associated antigens and antibodies making up these complexes; (3) determine the immune specificity of antibodies isolated from the effusion fluids; (4) detect tumor-associated antigen in the effusion fluid and tumor extracts; (5) utilize the tumor-specific antibodies to prepare an immunoadsorbent for the purification of the antigen; and antibodies to prepare an immunoadsorbent for the purification of the antigen; and (6) develop a diagnostic assay for detecting circulating antibody, antigen and antigen-antibody complexes in patients with various stages of ovarian malignancy.

Progress: Antibodies have been isolated from the effusion fluids of 38 patients with ovarian carcinoma. These antibodies have been absorbed with a polymer of normal ovaries and plasma, and tested for titer and specificity by indirect immunofluorescence (IF) against cells obtained from malignant effusions from ovarian and control cancer patients. Antibodies from 6 patients have shown specificity for ovarian cancer cells. Three approaches are being used to isolate an ovarian-associated antigen: (1) High titer absorbed ovarian antibodies coupled to Sepharose 4B have been used to isolate minute amounts of material from tumor extracts. (2) Preliminary results using Clq to test for immune complexes indicate that some of the fluids and also the antibodies isolated from them contain Clq-positive material. (3) Isolated IF-negative antibodies and/or complexes, when subjected to low pH gel chromatography or rapid neutralization and ion-exchange chromatography, have yielded antibodies which give positive results when tested by indirect IF against ovarian cancer cells. Material obtained by these approaches is presently being investigated for the presence of an ovarian-associated antigen(s).

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 12/13/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $190,992

CONTRACT RESEARCH SUMMARY

Title: Improving X-ray Imaging, Contrast, and Sharpness While Retaining Film Resolution

Principal Investigator: Dr. Gary T. Barnes
Name/Address University of Alabama at Birmingham
Performing Organization: Birmingham, AL

Contract Number: NO1-CB-53865
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To remove scatter and thus improve sharpness and contrast to improve the ability of recognizing tissue differences and thus identifying cancer in its earlier (smaller) stage.

Approach: To construct a scanning multiple slit assembly that will function in a clinically useful range of exposure times and delivered dose to obtain the stated primary goals by mechanical means which eliminate the need for grids and air gaps. Methods will be developed to determine quality of the films produced as well as relative patient doses required in their production. To be included are realistic measurements of improvements in scatter to primary detected radiation as a function of exposure and subject parameters, in addition to evaluation of resolution, contrast and dose reduction.

Progress: Evidence has been collected that an array of long, narrow beam-defining slits scanning a patient, coupled with scatter-eliminating slots beneath the patient, will substantially reduce scatter in diagnostic radiology. Such a device was developed and constructed at the University of Alabama at Birmingham. During an x-ray exposure the slots are synchronously moved with the beam-defining slits resulting in a uniformly exposed image receptor. The arrangement, which is called a Scanning Multiple Slit Assembly (SMSA), was shown to be substantially better than conventional grid scatter elimination techniques. When this device is employed in examining patients, superior x-ray films with no increase in patient exposure are expected.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 4/22/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $63,542

CONTRACT RESEARCH SUMMARY

Title: Detection of Pancreatic Cancer at Early or Small Stage Prior to Metastasis

Principal Investigator: Dr. A. R. Moossa
Name/Address
Performing Organization: University of Chicago
Chicago, IL

Contract Number: NO1-CB-43976
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: To evaluate diagnostic methods and findings in patients undergoing abdominal exploration for pancreatic cancer to establish a base that can be used to detect small pancreatic cancer before metastasis that may lead to an increase in the 5-year survival from 1% now prevalent to 13% or more.

Approach: Using a common protocol, three institutions will study patients suspected of having pancreatic cancer and about to undergo abdominal surgery. Studies will include preoperative and operative study of CEA in peripheral and portal venous blood. Pancreatic secretions will be studied for tumor-associated antigens, abnormal enzyme content, biochemical and cytological changes.

Progress: To date, 148 patients have been referred to this study. Of these, five patients had positive diagnosis of pancreatic cancer elsewhere and came to the hospital for radiotherapy and/or chemotherapy treatments. Systemic blood was taken, but no additional investigation was performed. Of the 143 patients entered into the study, 73 have been fully, and 70 partially, investigated. Of the 73 who completed the protocol, 50 have undergone laparotomy, and 24 pancreatic cancers were found. Of the 23 patients who completed the protocol and did not undergo laparotomy, none has developed pancreatic cancer, and all have been followed up from 6 months to 2 1/2 years. Of the 70 who were partially investigated, 46 underwent laparotomy, and 17 pancreatic cancers were found. The remaining 24 patients who were partially investigated did not undergo laparotomy, and two of them had pancreatic cancer diagnosed by biopsy and confirmed by autopsy.

On the basis of this study, the rational sequence of testing appears to be duodenal drainage studies, abdominal ultrasound, and liver scanning in the first instance. Patients with any documented abnormality and without evidence of metastases should undergo ERCP and arteriography. A pancreatic oncofetal antigen was partially purified and characterized and a quantitative rocket immunoelectrophoresis was developed to study its distribution in patients with various disorders. A high incidence of carbohydrate intolerance in patients with pancreatic cancer was demonstrated and a substantial decrease in insulin release following a standard glucose load in these patients was established.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/22/77
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $171,727

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Cooperative Lung Cancer Groups

Principal Investigator: Dr. C. Ralph Buncher
Name/Address University of Cincinnati Medical Center
Performing Organization: Cincinnati, OH

Contract Number: NO1-CB-43868
Starting Date: 6/25/74 Expiration Date: 5/31/78

Goal: To collect and analyze data from the three collaborating lung cancer groups in the lung cancer screening program directed toward the detection of early lung cancer in high risk patients to see if this screening will reduce mortality and morbidity.

Approach: Procedures have been established and agreement has been reached concerning the common data base for this study. The data are being monitored by the Statistical Center, entered into the computer and analyzed. Reports are being made quarterly. The Statistical Center will search the data for significant statistical findings.

Progress: In the Cooperative Early Lung Cancer Group program, the Cincinnati Central Statistical Group (CSG) has established a common data base, made suggestions for uniform data recording by the clinical centers, and reported results to the NCI and participating clinical centers. The CSG receives patient data from each of the clinical centers and forms a master patient file from which tables, reports, and analyses can be derived to answer the questions of the study, i.e., whether efforts directed toward earlier diagnosis have successfully led to improvement in mortality from lung cancer.

Tables showing the results and status at each of the clinics are produced. They have proven of value to the NCI and other participants as a means of monitoring the progress and results of the study. Patients' status indicator codes and information on follow-up have been added to the common data base, and computer programs for editing submitted information have been developed. In the past year, programs to calculate person-years of risk were developed and mortality records have been computerized.

This contract includes similar work in a three-center study of pancreatic cancer and a five-center study of brain cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $225,089

CONTRACT RESEARCH SUMMARY

Title: Detection of Small Amounts (over 7-10 ml/24 hours) of Human Blood in Human Feces

Principal Investigator: Dr. Curtis L. Songster
Name/Address: University of Louisville Foundation, Inc.
Performing Organization: Louisville, KY

Contract Number: NO1-CB-43968
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 ml in 24 hours) in human fecal material by a simple, accurate (98%) examination.

Approach: The contractor shall develop a procedure for the detection of human blood in feces by utilizing antigens specific for the globin portion (alpha and beta chains) of hemoglobin. Application of the test will be made to an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry, and fish diets and be truly negative by the proposed test.

Progress: High titer antisera have been raised against alpha and beta chains of human hemoglobin. Cow, pig, sheep, chicken, horse and fish hemoglobins lack cross-reaction. Cross-reactions with human hemoglobins are complete for A/A, A/S, A/C, S/S, S/C and monkey; partial with rabbit hemoglobin. Although native rabbit hemoglobin is weakly cross-reactive, heat-denatured rabbit hemoglobin (mimicking cooking of meat) is totally non-reactive.

Radial immunodiffusion techniques using anti-alpha-chain and anti-hemoglobin appear capable of specifically detecting human blood at levels of 2 mg% in purified A/A hemoglobin hemolysates. Fabricated fecal bleeds representing 3 to 100 milliliters of whole blood (assumed minimum hemoglobin levels of 10 grams/100 ml) can be routinely detected in 500 grams of stool sample thought to represent maximum fecal mass per 24-hour collection. Further antibody purification eliminated nonspecific precipitation. Various carriers and bacterial inhibitors have been employed to reduce bulk and contamination. Current studies are evaluating time, temperature, pH and bacterial flora effects upon the "antigen-carrier system." Test system components have a 90-day shelf life. Methods to increase sensitivity are being evaluated. Multiple specimen testing of preoperative patients with documented lesions is being studied to determine amount and frequency of bleeding, as well as locality of blood in the specimen.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 4/13/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $81,129

CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool to Detect Early Cancer of Bowel

Principal Investigator: Dr. Victor A. Gilbertsen
Name/Address University of Minnesota
Performing Organization: Minneapolis, MN

Contract Number: NO1-CB-53862
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: The goal of this project is to demonstrate a significant reduction in mortality due to colorectal cancer between the annually screened and the control group by employing the Hemoccult (R) form of the guaiac test for stool blood in combination with a diagnostic protocol for the source of bleeding.

Approach: Forty-five thousand participants between the ages of 50 and 80 years residing in the state of Minnesota are randomly allocated to three groups on the basis of age, sex, and geographic location in the state. Slides are completed after the observance of a meat-free diet with suggested high fiber content. One of the groups completes the slides once per year, another every other year, and a third group serves as the control. Participants submitting samples positive for blood receive a diagnostic examination (including a colonoscopy) to determine the cause of bleeding. All diagnostic procedures are conducted at the University of Minnesota Medical Center. All data including pathology reports on biopsy material and surgical specimens are computerized for easy retrieval. Extensive follow-up procedures are designed to retain participation of all subjects throughout the five years of testing and an additional five years of follow-up. Extensive dietary and health history data are collected from each subject via questionnaire.

Progress: Response rate after the first year of testing is 85% for test slides and 90+% for questionnaires (control group). Tested groups receive questionnaire at time of notification of negative slide results or at the time of diagnostic procedure should test be positive. Seventy-one percent of the 45,000 participants have been recruited as of May 1977.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 4/20/76
Technical Review Group: Diagnosis Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $609,000

CONTRACT RESEARCH SUMMARY

Title: Development and Serum Isozyme Test for Human Cancer Patients

Principal Investigator: Dr. K. C. Tsou
Name/Address University of Pennsylvania
Performing Organization: Philadelphia, PA

Contract Number: NO1-CB-64029
Starting Date: 6/30/76 Expiration Date: 4/30/78

Goal: To improve on the early diagnosis of cancer of the liver and further examine the possibilities of preventing the spread of primary cancers to other body sites with concentration on liver tissue.

Approach: The project involves: (1) further technical improvement of the 5'-nucleotide phosphodiesterase isozyme method by gel electrophoresis as a diagnostic tool; (2) further development of correlation data (biopsy, scan) and 5'-nucleotide phosphodiesterase-V for primary hepatoma; (3) further definition of possible relationships of the isozyme band V of this enzyme to liver metastasis; (4) investigation of possible relationships between this isozyme and other known liver markers related to hepatic cancer, especially α-fetoprotein and hepatitis B surface antigen (Australia antigen); (5) improvement of sensitivity of the test through development of an alternate assay method, including immunoassay if an antienzyme antibody can be prepared with reasonable ease; (6) correlation of clinical information and ôther liver function tests with the results of this test; and (7) protocol for the use of this test in development of an early diagnosis.

Progress: Out of 21 cases of biopsy proven primary hepatomas, 20 cases are positive for 5'-nucleotide phosphodiesterase isozyme-V (95%). Of this group, only 5 have α-fetoprotein level > 40 ng/ml. The one single case is negative for the isozyme and < 10 ng/ml for αFP. In the group of patients with known liver metastasis by biopsy or scan, 52 out of 54 samples are positive (96.47%). In the group of patients with no known liver metastasis at the time of sampling by either liver scan or clinical symptoms, 74/229 patients are positive (32.3%). Follow-up of 11 patients in this group showed that eight did have metastasis to the liver in their autopsy record.

This isozyme has also been found in active hepatitis B and disappears when the patient recovers. Australian antigen-positive carriers are, however, negative with respect to this isozyme.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 3/4/77
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $110,043

CONTRACT RESEARCH SUMMARY

Title: Experiment to Assess the Impact of Multi-site Screening on Total Cancer Mortality

Principal Investigator: Dr. Dean F. Davies
Name/Address University of Tennessee
Performing Organization: Memphis, TN

Contract Number: NO1-CB-53864
Starting Date: 6/23/75 Expiration Date: 6/22/77

Goal: The goal of this project is to achieve a design that can be put to clinical use in order to effectively reduce cancer mortality.

Approach: A team has been established with expertise in the fields of clinical oncology, radiology, epidemiology, and statistics. The experimental design will be developed on the basis of available knowledge of (1) high-risk factors, (2) quality of screening procedures, (3) variability in the effectiveness of present day treatment, (4) cost, (5) compliance with follow-up examinations and follow through procedures, (6) side effects of treatments, (7) incidence rates, (8) prevalence rates, and (9) case fatality rates. The rationale for the experimental design will be expressed in a format which makes explicit the use of available information and accepted assumptions.

Progress: A randomized experiment has been designed for determining the effectiveness and the cost-benefit of multi-layered screening for lung, breast, colon/rectum, cervical and endometrial cancers. For this purpose a simulation model has been constructed and tested, and the optimum age groups, optimum risk levels, markers, and end-points have been specified.

By judicious prescreening by questionnaire the composite risk levels that are necessary to exclude the low-risk populations and to obtain a more cost-effective experiment using relatively high yield populations is possible. The experiment would test the validity and precision of the risk level estimates.

Acquisition of cost estimates and completion of the protocol of the design should be completed within the next year. Pretesting of selected "nodes" of the Flow Tree Diagram is indicated for a reliable estimate of the magnitude and cost of the experiment.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 5/4/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $107,250

CONTRACT RESEARCH SUMMARY

Title: Improvement of X-ray Imaging by Elimination of Scatter While Retaining the Resolution

Principal Investigator: Dr. Melvin P. Siedband
Name/Address University of Wisconsin
Performing Organization: Madison, WI

Contract Number: NO1-CB-53914
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: To remove scatter and thus improve sharpness and contrast which will in turn improve the ability of recognizing tissue differences and thus identifying cancer in its earlier (smaller) stage.

Approach: To construct a system that will function in a clinically useful range of exposure times and delivered dose and that can obtain the stated primary goals by mechanical and/or electronic means and should eliminate the need for grids and air gaps. Methods will be developed to determine the quality of the films produced as well as relative patient doses required in their production. To be included are realistic measurements of improvements in scatter to primary detected radiation as a function of exposure and subject parameters, in addition to evaluation of resolution, contrast and dose reduction. Feasibility should be established during the first year of this contract.

Progress: (1) A servo-coupled moving paired-slit system was constructed and tested as a preliminary to the designing of single or multiple-slit systems. (2) A two-color cassette and beam filter have been developed. The cassette incorporates two screens, each having different energy sensitivities and which excite the two emulsions of the film. (3) Preliminary tests have been made with a differential absorption cassette designed for detection of breast calcifications. The cassette uses mammographic screens, and the 60 kVp x-ray beam was filtered with iodine to give 2 peaks with effective energies of about 30 and 45 keV. Radiographs of the Kodak Pathe mammography phantom were made which showed good soft tissue subtraction, but with low calcification contrast level. We are now experimenting with filters having K-edges at energies lower than that of iodine (33 keV), in order to raise the contrast level.

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: Louis P. Greenberg
Program: Diagnosis Site Visit Date: 4/21/76
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $77,138

The Committee on Cytology Automation was established in July, 1972 to advise the Director, Division of Cancer Biology and Diagnosis, National Cancer Institute on all matters pertaining to Cytology Automation. Specifically, the Committee (1) serves as advisors to National Cancer Institute on the state of the art in scientific and technical areas which bear on automation, and (2) advises the National Cancer Institute on plans and implementation, in operational terms for National Cancer Institute's contractual support of developing automation in cytopathology.

The Committee determined that six areas are within the mission of the Committee:

1. Specimen Collection
2. Specimen Preparation
3. Sensing-screening Systems
4. Decision-diagnostic Systems
5. Specimen Sources
6. System Evaluation

At the present time several very important areas of research have been accomplished. In area 1, specimen collection, it has been determined in an extensive study by Dr. Marluce Bibbo and collaborators at the University of Chicago that the number of single abnormal cells present in gynecologic specimens is adequate for screening purposes in essentially all cases of pre-malignant and malignant lesions. Thus only relatively gentle disaggregation procedures are necessary in preparing clinical specimens for automated analysis.

In area 2, specimen preparation, a thorough examination of mechanical and enzymatic dispersal procedures to increase the yield of single intact cells has been completed by several contractors. The results of this work indicate that relatively simple mechanical dispersal techniques such as pressure-controlled syringing and ultrasonic treatment produce adequate numbers of morphologically and cytochemically intact cells for instrumental analysis.

In areas 3, 4, and 6, development and evaluation of integrated screening systems, several automated analytic systems have been identified as promising potential designs for automated clinical prescreening systems. Evaluation and development of these systems is proceeding to, a) ensure that they reflect a sound biologic basis for identification of pre-malignant and malignant squamous cell lesions of the gynecologic tract; b) evaluate their performance characteristics, especially preliminary analysis of sensitivity and specificity.

Work is continuing on the identification of quantitative descriptors of pre-malignant and malignant lesions, especially cytochemical and immunological

markers which will increase the potential sensitivity and specificity of currently employed sensor systems in screening clinical material. In addition, research is in progress to develop and test a new sorter to separate cells based on individual cell parametric measurements assuring a correlation of individual separated cells with specific quantitative measurements in flow systems. Research has begun to develop and test single cell classification algorithms employing the current state of the art in digital image processing, scene segmentation and image acquisition hardware. Work has also been initiated in the comparison of instrumental or system classification and microscopic "true classification" utilizing standard morphologic criteria. The data will have a direct bearing on the analysis of the performance of any automated systems.

CONTRACT RESEARCH SUMMARY

Title: Development of New Methods of Single Cell Separation

Principal Investigator: E. Robert Schildkraut
Name/Address Block Engineering, Inc.
Performing Organization: Cambridge, MA

Contract Number:
Starting Date: Expiration Date:

Goal: Development and testing of single cell separator that will reliably associate individual separated cells with specific quantitative measurements.

Approach: The contractor shall develop and analyze techniques for deposition, fixation, staining and scribing system for gynecologic cells on film. The speed and fluid volumes will be compatible with the output of a flow microfluorometric system. The design of this scribing system will not be such as to preclude its adaptation as a module to presently available FMF systems other than the contractor's. The contractor shall also develop and analyze a fiducial system which will allow reliable unique recall and identification of individual cells.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of a reliable clinical screening system depends on the identification and assurance that "abnormal" quantitative signals are associated with abnormal cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 2/9/77
Technical Review Group: Cytology Automation Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $300,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Application of Flow System Methods of Cell Analysis and Sorting to Cancer Screening

Principal Investigator: Dr. Paul Mullaney
Name/Address ERDA -- Los Alamos Scientific
Performing Organization: Laboratory
Los Alamos, NM

Contract Number: NO1-CB-60311
Starting Date: 4/1/71 Expiration Date: 5/31/78

Goal: Automation of clinical cytology screening and diagnosis.

Approach: The contractor shall (1) collaborate with Battelle Institute to study light scattering and diffraction, (2) evaluate improved methods of data acquisition and analysis applicable to multiparameter flow systems, (3) implement and test a dual laser sorter using an added UV krypton laser and new stains, and (4) evaluate improved computer interface for existing sorter.

Progress: The contractor has obtained a large number of gynecologic specimens for which some data, fixation, and staining techniques have been developed. The acriflavin-feulgen procedure for DNA staining, mithramycin as a rapid DNA stain, propidium iodide and fluorescein isothiocyanate as a simultaneous DNA-protein staining technique, narrow angle light scatter, multi-angle light scatter, time-of-flight, and Coulter volume parameters have been implemented on flow systems. These show high potential as useful parameters in gynecologic cancer screening. The contractor has also made major improvements in the electronic cell sorting technology which will be used to demonstrate the correlation between these parameters and the morphology. A dual laser sorter has been constructed and will be tested using a number of fluorochromes. Improved computer interface is undergoing analysis. The collaborative work with Grumman Aerospace Corporation has been concluded.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 1/5/77
Technical Review Group: Cytology Automation Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $370,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Vaginal-Cervical Sample Sources

Principal Investigator: Dr. Gregorie Delgado
Name/Address Georgetown University Hospital
Performing Organization: Washington, DC

Contract Number: NO1-CB-64015
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To supply NCI and contractors with samples to evaluate their experiments and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology 1200 gynecologic cytopathology samples obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate instrumentation and methods of sample preparation and staining, both at NCI and at other contracting institutions within this program.

Progress: The contractor has delivered to NCI gynecologic cell samples ranging from normal to invasive squamous cell carcinoma. These samples are being used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter. A number of these samples have been sent to other contractors for use in their studies.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $42,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Dr. Raymond Wall
Name/Address
Performing Organization: JPL -- California Institute of Technology
Pasadena, CA

Contract Number:
Starting Date: Expiration Date:

Goal: Development of optimal algorithms for cell classification applicable to automated digital image processing system.

Approach: The contractor shall determine whether or not current state of the art in pattern recognition is adequate to support the development of an economically viable cytological screening instrument based on single cell classification. Specimen preparation protocol will be optimized so as to produce acceptable single cells for digitization. A large number of digitized images will be accumulated in the library for subsequent feature extraction program to produce a feature data base. A series of classification experiments will be run on the feature data base to select the subsets of cell measurements that yield the best overall performance.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Single cell classification algorithms are the key to success in any automated digital image processing system applied to cytology automation.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 5/23/77
Technical Review Group: Cytology Automation Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $200,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Markers for Gynecologic Specimens

Principal Investigator: Dr. Torbjorn Caspersson
Name/Address
Performing Organization: Karolinska Institute
Stockholm, Sweden

Contract Number: NO1-CB-43945
Starting Date: 6/30/74 Expiration Date: 6/29/78

Goal: Automation of clinical cytologic screening and diagnosis, concomitant with improvement in diagnostic accuracy and throughput.

Approach: Dr. Caspersson has pioneered the development of innovative methods of investigative cytology and cytogenetics. Under this contract, he will develop and evaluate prototype machines for automatically measuring cytologic features of clinical gynecologic specimens using high resolution scanning microscopy with absorption ultraviolet-spectrophotometry, interferometry and cytofluorometry, individually and in combination. Quantitative measurements of the various cell types of the female genital tract will yield multiparameter data which can be used as discriminators for these different cell types by automated cytology instruments.

Progress: A two-parameter instrument has been constructed. The instrument consists of computer-controlled servomotor driven scanning microscope, microspectrophotometer and microinterferometer. The scanning microscope is used to examine and select cells and has the capability to recall the previously chosen cells for reexamination and analysis. The integrated instrument permits microspectrophotometric measurements in the UV and/or visible range, followed by microinterferometric measurements on the same cell while the cell remains in the same microscope field of vision. Modification and improvement of the systems including incorporation of image intensifier-camera system have been completed. Data on gynecologic materials are being accumulated and analyzed.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Development of very rapid high resolution multiple modality automated scanning microscopes will advance image processing approaches for cytologic screening and diagnosis, and promote better understanding of normal, precancerous and cancerous states.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 6/26/76
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $46,000

CONTRACT RESEARCH SUMMARY

Title: New Stains and Other Optical Markers Useful for Gynecologic Specimens

Principal Investigator: Dr. Brian H. Mayall
Name/Address: Lawrence Livermore Laboratory
Performing Organization: Livermore, CA

Contract Number: YO1-CB-40300
Starting Date: 6/1/74 Expiration Date: 6/29/77

Goal: Development and preliminary clinical testing of stains useful in differentiating normal from non-normal cytopathological material.

Approach: Utilizing the unique combination of flow-sorting and image-processing expertise, the contractor will evaluate specimen preparation and staining techniques on clinical samples. The ability to evaluate specimens at both zero and medium to high resolution will assure the mutual compatibility of preparative techniques and safeguard the diagnostic value of samples prepared for automated systems.

Progress: Clinical specimens have been collected and used for production of monodisperse cell suspensions. The cells are treated by mechanical means, e.g., syringing and chemicals, and by enzymes and toxins. Cells of various types have been photographed and mapped for quantitative cytochemistry. The approach ensures that specific cell types are analyzed, as the CYDAC is employed to digitize and compile data on these cells. Correlation between data and selected cells can be made since each cell was previously photographed and recorded. Cells in suspension are also quantitatively analyzed by the LLL flow microfluorometer. Data have been obtained pertaining to different fluorescent properties of the various cell types. More samples are being analyzed. Several fluorochromes have been evaluated for their potential usefulness in cell differentiation. These include: Azure A-Fuelgen, naphthol yellow S, BMB (4-bromomethyl-7-methoxy-2-oxo-2H-benzpyran), and chromomycin A3. A dual laser flow system has been implemented and will be used to study cytochemical probes for chromatin structure.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Development and evaluation of techniques to rapidly screen cell populations for premalignant and malignant cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 5/14/76
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $390,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Development of Hematoxylin Substitutes for Staining of Tissues and Exfoliated Cells

Principal Investigator: Dr. Ralph D. Lillie
Name/Address Louisiana State University
Performing Organization: Baton Rouge, LA

Contract Number: NO1-CB-43912
Starting Date: 1/11/76 Expiration Date: 1/10/79

Goal: Development of hematoxylin substitutes for diagnostic staining of tissues and exfoliative cells.

Approach: (1) Synthesize various classes of mordant dyes or obtain same from either specific manufacturers or from the archives of the Biological Stain Commission. (2) Perform studies on various tissues with combinations of dyes obtained to achieve staining in accordance with criteria satisfactory to the Project Officer and to the Biological Stain Commission's certification criteria. (3) Procure samples of possibly suitable mordant dyes offering blue or black mordant colors which may be suitable to replace hematoxylin in one or more of its histological staining uses. (4) Subject such samples to the usual tests applied by the Biological Stain Commission for the certification of hematoxylin. (5) Apply favorable test results to routine surgical diagnostic material, in Papanicolaou smear diagnosis, and in other staining procedures applicable to the differential diagnosis of various cancers, evaluating results in each case in comparison of previously standard hematoxylin procedures and with the best of the previously proposed substitutes.

Progress: Synthesis and testing are on schedule as outlined above.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Stain development is essential to the cytological diagnosis of cancer.

Project Officer: M. F. Stanton, M.D.
Program: Cytology Automation
Technical Review Group: Ad Hoc Committee Laboratory of Pathology
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $48,911

CONTRACT RESEARCH SUMMARY

Title: Methods for Obtaining Monodisperse Suspensions of Cytology Specimens

Principal Investigator: Dr. Leopold G. Koss
Name/Address Montefiore Hospital and Medical Center
Performing Organization: Bronx, New York

Contract Number: NO1-CB-43963
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: To obtain monodisperse cell suspensions of gynecologic cytopathology specimens for use in flow-through systems being developed for cytology automation.

Approach: The principal investigator will study the natural cell attachments and various ways of disrupting these attachments. Electron microscopy will be used to evaluate the effectiveness of the cell attachment (chiefly desmosome) disruption.

Progress: The contractor has successfully evaluated a number of methods of cell disaggregration and their relative cell loss. Nuclear isolation experiments were conducted and the results evaluated by flow microfluorometric analysis. Studies into means of isolating desmosomes from human cervical and vaginal material were conducted. Desmosome formation has been studied in tissue culture models.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 11/18/75
Technical Review Group: Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Furnishing a Cytoscreener and Technician for Testing Purposes

Principal Investigator: Melvin P. Ehrlich
Name/Address
Performing Organization: Nuclear Leasing Corporation
Hyde Park, NY

Contract Number: NO1-CB-53915
Starting Date: 6/1/75 Expiration Date: 4/30/77

Goal: To supply Cytoscreener for engineering and biological evaluation.

Approach: The Cytoscreener, manufactured and patented by Nuclear Research Associates, has been proposed as an instrument suitable for automation of clinical cytologic screening. Clinical tests have been equivocal. Basic engineering and biologic data are needed before further clinical testing can be conducted.

Progress: The Cytoscreener has been successfully evaluated. The evaluation included the instrument's subsystems and performance using specific test samples.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 10/11/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $27,248

CONTRACT RESEARCH SUMMARY

Title: New Stains and Other Optical Markers for Clinical Specimens in Suspension

Principal Investigator: Dr. Robert C. Leif
Name/Address
Performing Organization: Papanicolaou Cancer Research Institute
Miami, FL

Contract Number: NO1-CB-43962
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: Investigation of new markers and techniques of specimen preparation for clinical cytopathologic screening.

Approach: The investigator will extend the technique of centrifugal cytology to produce improved specimens for analytic instrumentation. Techniques for dialysis staining, heavy metal chelating fluorochromes and immunofluorescence will be investigated as preliminary feasibility studies.

Progress: Dialysis staining machines and centrifugal cytology buckets have been constructed and modified. Cell dispersion studies have been concluded; enzymes and chemicals tested appear to produce cervical, vaginal cell morphologic damage. The value of cervical carcinoma antigens is being further assessed for possible use as abnormal cell markers. The synthesis of rare earth chelates has continued.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of new markers and preparative techniques for clinical specimens will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 6/9/76
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator:	Dr. John J. Docherty
Name/Address	Pennsylvania State University
Performing Organization:	University Park, PA
Contract Number:	
Starting Date:	Expiration Date:

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: The contractor shall isolate "tumor antigen" extracted from HSV-1 and HSV-2 transformed cells, and prepare corresponding purified antisera for labeling with peroxidase and fluorescein isothiocyanate and testing on gynecologic exfoliated cervical cells and biopsy specimens. Cell by cell analysis will be done to assess the sensitivity and specificity of the assay. Techniques for immunofluorescent staining of exfoliated cells in suspension will be developed for use with flow microfluorometric cell sorter.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $105,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Methods for Obtaining Monodisperse Cells and Markers for Gynecologic Specimens

Principal Investigator: Dr. Paul Todd
Name/Address Pennsylvania State University
Performing Organization: University Park, PA

Contract Number: NO1-CB-43984
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: Investigation of new techniques for preparing and marking cells for automated prescreening of clinical cytology specimens.

Approach: Cell electrophoresis, both micro and bulk, will be investigated. Potential utility of HSV-II antigens in distinguishing normal from abnormal human cells will be explored. A number of enzymatic techniques for cell dispersal will be investigated and analyzed.

Progress: Cell electrophoresis studies have been concluded. At present, analytical electrophoresis for distinguishing normal and abnormal cells and for preparative separations of the gynecologic cell types is not feasible. The data on the cell dispersion confirm earlier findings that automatic pressure controlled syringer produce the least damaged single cells and that enzymatic and chemical treatments of gynecologic specimens induce morphologic damage. HSV-II non-virion antigens are being further explored as possible markers for abnormal cells.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Cell preparative techniques and exploitable markers hold the key to automation of cytology screening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 6/11/76
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Markers for Detection of Abnormal Cervical Cells by Optical Methods

Principal Investigator: Dr. B. D. Halpern
Name/Address Polysciences, Inc.
Performing Organization: Warrington, PA

Contract Number: NO1-CB-43943
Starting Date: 6/1/74 Expiration Date: 5/31/77

Goal: Chemical synthesis and preliminary testing of markers for clinical specimens.

Approach: The contractor will produce absorptive and fluorescent dyes to stoichiometrically quantitate macromolecules, enzymatic activity and antigen-antibody systems suggested by basic scientific research to be of potential utility in distinguishing human malignant and premalignant cells from non-neoplastic cells in automated cytopathologic screening instruments.

Progress: The Contractor has produced various sized fluorescent-labeled microspheres with functional groups. The contractor has also collaborated with other contractors in conjugation of microspheres, rare earth chelate synthesis, and purification of several fluorochromes.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Chemical synthesis and verification of stoichiometric stains useful for clinical use in screening cytopathology specimens.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 10/25/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Dr. James W. Bacus
Name/Address Rush-Presbyterian-St. Luke's
Performing Organization: Medical Center
Chicago, IL

Contract Number:
Starting Date: Expiration Date:

Goal: Development of optimal algorithms for cell classification applicable to automated digital image processing system.

Approach: The contractor shall conduct a comprehensive study to determine single cell vs. specimen classification accuracies for image processing algorithms and for cytotechnologist. This will determine how well the machine algorithms are performing. Five experimental tasks will be done: (1) sample acquisition, (2) cell acquisition, (3) observer recognition, (4) cell classification algorithms development, and (5) analysis and evaluation of results.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Single cell classification algorithms are the key to success in any automated digital image processing system applied to cytology automation.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 5/30/77
Technical Review Group: Cytology Automation Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $230,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Methods for Obtaining Monodisperse Preparations of Cytology Specimens

Principal Investigator: Dr. Walter E. Tolles
Name/Address State University of New York
Performing Organization: Brooklyn, NY

Contract Number: NO1-CB-43955
Starting Date: 6/1/74 Expiration Date: 5/31/78

Goal: To obtain a monodisperse cell sample from gynecologic cytologic specimens for use in flow-through and image processing systems being developed for cytology automation, and to supply NCI and other contractors with treated and untreated gynecologic samples for use in the evaluation of sample preparation and instrumentation.

Approach: The emphasis will be on mechanical disaggregration and enrichment techniques. Chemical and enzymatic agents will be used in combination with ultrasound to break up cell clusters. Rate zonal sedimentation will be used as an enrichment technique. The results of the various experiments will be evaluated microscopically with emphasis on disaggregration of clusters and cell damage. These samples will be sent to NCI and other contractors in this program.

Progress: Ultrasound appears to be the most effective means of disaggregation for this contractor. An improved ultrasonicator has been developed so the energy output can be monitored. A new sedimentation chamber has been designed and tested. Other aids to sample preparation are being evaluated, such as automatic slide making and nephelometry which gives an indication of the cellular content of the samples before processing. The gynecologic cytology samples which have been delivered to NCI have been adequate in diagnostic cellular material. A portion of these samples has been sent to other contractors for use in their studies.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 11/19/75
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $142,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigator:
Name/Address
Performing Organization:

Dr. Earl Greenwald
Temple University Medical School
and Hospital
Philadelphia, PA

Contract Number: NO1-CB-43937
Starting Date: 6/30/74
Expiration Date: 6/29/78

Goal: To supply NCI and contractors with samples to evaluate their experiments and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology 600 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: The contractor has delivered to NCI gynecologic cell samples ranging in diagnosis from normal through invasive squamous cell carcinoma. These samples are being used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter. A number of these samples have been sent to other contractors for use in their studies.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Site Visit Date: 7/11/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $24,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Dr. Laure Aurelian
Name/Address The Johns Hopkins University
Performing Organization: School of Medicine
Baltimore, MD

Contract Number:
Starting Date: Expiration Date:

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: The contractor shall isolate HSV antigens: (i) Total viral antigens, (ii) AG-e and (iii) fractions of AG-e, and prepare corresponding purified antisera to test sensitivity and specificity of these antisera to human gynecologic materials. Immunological and biochemical characterization of the antigens will be done.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 2/24/77
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $75,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Stains Useful for Gynecologic Specimens

Principal Investigator: Dr. Seymour West
Name/Address University of Alabama
Performing Organization: Birmingham, AL

Contract Number: NO1-CB-43960
Starting Date: 6/30/74 Expiration Date: 6/29/77

Goal: Evaluation of the applicability of biophysical instrumental analysis to clinical cytopathologic pre-screening.

Approach: The contractor will apply biophysical techniques to evaluate acridine orange as a molecular probe and fluorescence fading phenomenon.

Progress: Nuclear fluorescence intensity of fixed cells from clinical gynecologic specimens and acridine orange fluorescent fading of nuclear macromolecules are under investigation.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
Identification of new machine sensible properties suitable for distinguishing neoplastic from non-neoplastic human cells.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 11/22/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Image Processing for Development of Automated Cell Recognition System

Principal Investigator: Dr. George L. Wied
Name/Address University of Chicago
Performing Organization: Chicago, IL

Contract Number: N01-CB-33873
Starting Date: 5/31/73 Expiration Date: 4/30/78

Goal: Automation of clinical cytologic screening and diagnosis, focusing on definition of information requirements and criteria for high throughput, high resolution image processing-based decision making.

Approach: The contractor shall evaluate information requirements for characterization and recognition of normal, premalignant and malignant cells from the human female genital tract by means of digital image processing. Robustness of cytologic features and discrimination logic will be assessed as a function of minor variations in staining and fixation procedures.

Progress: Digitized cell images have been compiled. These data are stored on tapes and will be made available to other contractors for further analyses. These cells are from the various cell types encountered in the exfoliated cell specimens from the female genital tract. Image editing system is operational. Sorted cleaned cell images are available for other contractors to analyze with their own algorithms. Further details on scanned cells are being done using 3-color scanner. New computer program has been written to evaluate the new information. The multi-spectral band scanner has enriched the data base for cell image processing. The new color features enhance the classification of metaplasias, dysplasias and malignant cases. Feature extraction for chromatin distribution patterns has been implemented. High-resolution data acquisition module and Cell Detection Module hardware are being implemented.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
A rapid, high-resolution, multi-spectral digital scanner for clinical gynecologic screening and diagnosis will promote better health care delivery and therapy monitoring for the populations at large and at high risk.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 6/8/76
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $234,539

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Dr. Harold Haines
Name/Address
Performing Organization: University of Miami School of Medicine
Miami, FL

Contract Number:
Starting Date: Expiration Date:

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: Contractor shall isolate and purify cervical carcinoma antigen (CCA). Appropriate antisera to CCA will be prepared and cell by cell analysis using indirect immunofluorescent technique and restaining by Pap staining procedure will be done. Exfoliated cells, tissue imprints and tissue sections will be used. Technique for immunofluorescent staining of exfoliated cells in suspension will be developed for use in flow microfluorometric cell sorter.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $120,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Slit-Scan Technique as Cancer Prescreening Automated System in Cytology

Principal Investigator: Dr. Leon L. Wheeless, Jr.
Name/Address University of Rochester
Performing Organization: Rochester, NY

Contract Number: NO1-CB-33862
Starting Date: 3/19/74 Expiration Date: 3/18/78

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The contractor will evaluate the VIDICON imaging system. The system is interfaced to the slit-scan flow instrument as a means of imaging cells as they pass through the flow chamber. Causes of false alarms will be determined using this system and ways of eliminating them will be attempted.

Progress: A second bench mark study was completed to evaluate a new sample preparation technique. The false alarm rate was reduced considerably. Two images of an individual cell taken from different angles can now be correlated with that particular cell's slit-scan contour. In this manner cells which cause false alarm signals can be imaged and investigations into possible ways to eliminate these false alarms can be conducted.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The development of a flow-through screening system for cervical cytology will allow greater accuracy, speed and availability in screening and diagnosis of cancerous and precancerous lesions.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 6/23/75
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $368,741

CONTRACT RESEARCH SUMMARY

Title: High-Resolution Fluorescence Measurements and Quantitative Study of Fluorochromes

Principal Investigator: Dr. Leon L. Wheeless, Jr.
Name/Address University of Rochester
Performing Organization: Rochester, NY

Contract Number: NO1-CB-53930
Starting Date: 6/30/75 Expiration Date: 6/29/77

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The principal investigator will investigate "high-resolution" fluorescence measurements on cells from the human female genital tract. A quantitative study of acridine orange and other fluorochromes useful in automated cell analysis systems will be performed.

Progress: Work is continuing in the investigation of the staining properties of acridine orange. Measurements have been made on the nuclear fluorescence of normal and abnormal gynecologic cells stained with acridine orange. Nuclear absorbance measurements were made on aliquots from the same samples of cells stained by the Feulgen DNA technique. Ratios of the level of nuclear fluorescent emission of normal cells to abnormal cells was compared to the ratios of the level of DNA absorbance of normal cells to abnormal cells. The statistical distribution indicates that the ratios are different. Work will continue to explore this phenomenon.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: 4/26/76
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Engineering and Biological Operations of NLC Cytoscreener

Principal Investigators: Drs. L. A. Couvillon and Doran R. Klingler
Name/Address: Utah Biomedical Test Laboratory
Performing Organization: Salt Lake City, UT

Contract Number: NO1-CB-53867
Starting Date: 6/1/75 Expiration Date: 4/30/77

Goal: Engineering and biological evaluation of the operation of Cytoscreener.

Approach: The Cytoscreener has been proposed as an instrument suitable for automation of clinical cytologic screening. Clinical tests of the instrument have been equivocal. Basic engineering and biologic data are needed before further clinical testing can be conducted.

Progress: Two major subsystems of the Cytoscreener, imaging and pattern recognition, were analyzed. Flow system analysis, power supply tests and computer interface have also been concluded. The overall performance of the Cytoscreener was successfully evaluated using specific test samples of known parameters.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Project Officer: Bill Bunnag, Ph.D.
Program: Cytology Automation Site Visit Date: 4/22/76
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $23,959

CONTRACT RESEARCH SUMMARY

Title: Pre-Clinical Studies of Chemotherapeutic Studies for Mycosis Fungoides

Principal Investigator: Dr. R. M. Folk
Name/Address: Battelle Memorial Institute
Performing Organization: Columbus, Ohio

Contract Number: NO1-CM-43746
Starting Date: 3/1/75 Expiration Date: 2/29/76

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of mycosis fungoides, a cutaneous lymphoma.

Approach: To increase the knowledge of the pharmacology of chemotherapeutic agents in topical application, investigation of cutaneous irritation, and percutaneous absorption will be carried out.

Progress: Subcontract has been awarded to the University of Mississippi School of Pharmacy. Cutaneous irritation studies have been done. Reports to date indicate that a few chemotherapeutic agents are irritating to rabbit skin after a single application. Percutaneous absorption studies are in progress.

Significance for Cancer Research (NCP Objective 6 Approach 2)

Project Officer: Dr. Gary Peck
Program: Biology Support Site Visit Date:
Technical Review Group: Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 76 Funds: $130,000

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Mr. W. Bruce Ramsay
Name/Address
Performing Organization: Computer Services Division
National Bureau of Standards
Gaithersburg, Maryland

Contract Number: YO1-CB-80047
Starting Date: 7/1/73 Expiration Date: 9/30/76

Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach:

Progress:

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support Site Visit Date:
Technical Review Group: Ad Hoc
Relevance Review Group:
FY 78 Funds: $80,000

CONTRACT RESEARCH SUMMARY

Title: A Screening System for Topical Chemotherapy of Mycosis Fungoides

Principal Investigator Name/Address Performing Organization: Dr. Stanford Lamberg
Johns Hopkins University
Baltimore, MD

Contract Number: NO1-CB-63927
Starting Date: 6/30/76 Expiration Date: 6/29/78

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of mycosis fungoides, a cutaneous lymphoma.

Approach: Chemotherapeutic agents are obtained commercially and from the DCT, NCI, and these agents are being screened by clinical patch testing in patients with mycosis fungoides.

Progress: Investigative new drug applications have been obtained from the FDA and patch test screening of these agents has begun at Hopkins and other affiliated institutions comprising the Mycosis Fungoides National Cooperative Group.

Significance for Cancer Research (NCP Objective 6 Approach 2)

Project Officer: Dr. Gary Peck
Program: Biology Support Site Visit Date:
Technical Review Group: Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $93,539

CONTRACT RESEARCH SUMMARY

Title: Administrative Support Services

Principal Investigator: Ms. J. Aldridge
Name/Address Kappa Systems, Inc.
Performing Organization: Arlington, VA

Contract Number: NO1-CB-53866
Starting Date: 11/1/74 Expiration Date: 10/31/77

Goal: To support administrative staff in preparation of scientific meetings and the research components in accomplishing their administrative tasks.

Approach: Current staffing situation places impossible demands on the administrative staff and committee chairmen (presently non-NCI scientists) in carrying out their responsibilities. This project is to support such routine tasks.

Progress: Executive secretaries and senior staff members were assisted in organizing scientific meetings of various committees. This made it possible to handle effectively the volume of administrative burden without over-straining available resources. Committee meetings and three program overviews were organized during the contract year. On the intramural research level, routine administrative and support activities were handled with minimal disruption of innovative research. In particular, the contractor assisted the intramural staff in handling reprint service and in preparation of manuscripts.

Supportive background material was prepared for: Diagnosis Research Overview, Immunotherapy Conference and BCTC Report to the Profession - the "White House" conference. Assistance was rendered in preparation of the Annual Report for the Division.

Significance for Cancer Research (NCP Objective 5 Approach 1-7)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: DCBD Site Visit Date: 9/74
Technical Review Group: DCBD - Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY 77 Funds: $426,228

CONTRACT RESEARCH SUMMARY

Title: Development of a Version of the SAAM Program for the Control Data (CDC) Computer

Principal Investigator: Dr. D. Austin
Name/Address: Lawrence Berkeley Lab (ERDA/LBL)
Performing Organization: Livermore, CA

Contract Number: YO1-CB-60307
Starting Date: 7/1/76 Expiration Date: 9/30/76

Goal: Compiling, testing and maintaining the CDC version of the Simulating, Analysis and Modeling (SAAM) Program.

Approach: N/A

Progress: Conversion of SAAM 27 program for CDC computers completed.

Significance for Cancer Research (NCP Objective 4 Approach 4)

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support Site Visit Date:
Technical Review Group: Ad Hoc
Relevance Review Group:
FY 76 Funds: $1,653.00

CONTRACT RESEARCH SUMMARY

Title: Development of Hematoxylin Substitutes for Diagnostic Staining if Tissues and Exfoliated Cells

Principal Investigator: Ralph Lillie
Name/Address Louisiana State University
Performing Organization: Medical Center
New Orleans, LA

Contract Number: NO1-CB-43912
Starting Date: 1/10/74 Expiration Date: 1/10/79

Goal: Develop dyes that will adequately replace the conventional hematoxylin now used for routine staining of histological and cytological preparations.

Approach: Synthesize and discover by other means, potential dyes for this purpose. Test such dyes and review results.

Progress: Several reasonable substitutes are being tested exhaustively for compatibility with present systems. These include Phenocyamin TC and other orcein type preparations.

Significance for Cancer Research (NCP Objective____Approach____)
Innovative staining techniques for the examination of tissues are essential to better diagnostic methods.

Project Officer: Mearl F. Stanton, M.D.
Program: General Contract Site Visit Date:
Technical Review Group: Ad Hoc Technical Review Group on Histolog. Diagnosis
Relevance Review Group: DCBD Contract Review Board
FY 77 Funds: $46,362

CONTRACT RESEARCH SUMMARY

Title: Preparation and Analysis of Cell Surface Protein (CSP) Fractions

Principal Investigator: Dr. David Schlesinger
Name/Address Massachusetts General Hospital
Performing Organization: Boston, MA

Contract Number: NO1-CB-64079
Starting Date: 9/30/76 Expiration Date: 9/29/77

Goal: To prepare and analyze the structure of cell surface protein.

Approach: Cell surface protein has been converted into fragments by treatment with cyanogen bromide. The fragments are to be tested for their biological activity. CSP has also been treated with a reagent that reacts with cysteine residues to give other types of fragments. These fragments are also being prepared for testing for biological activity.

CSP has been shown to be absent in many kinds of cancer cells and its absence has been correlated with its tumorigenicity of such cells. The knowledge of the structure of CSP will further our understanding of the cancer process.

He will prepare many types of CSP fragments to determine the function of different regions of the molecule. He will then determine the amino acid sequence of these fragments.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Dr. Ira Pastan
Program: Laboratory of Molecular Biology
Technical Review Group: None
Relevance Review Group: DCBD Board of Scientific Counselors
FY 77 Funds: $57,480

CONTRACT RESEARCH SUMMARY

Title: Radioimmunoassay of Immunoglobulin Molecules

Principal Investigator: Dr. Kenneth Kortwright
Name/Address: Meloy Laboratories, Inc.
Performing Organization: Springfield, Virginia

Contract Number: NO1-CB-63932
Starting Date: 3/15/77 Expiration Date: 3/14/78

Goal: To perform radioimmunoassays of immunoglobulin molecules IgM, IgA, IgG, IgD, and IgE in lymphocyte culture supernatants or in other biological fluids.

Approach: The contractor is to perform determinations of IgG, IgA, IgM, IgD, and IgE by double antibody radioimmunoassay by procedures defined by the project officer and using reagents supplied by the project officer. This contract provides critically required research support for studies on the nature of immunodeficiencies that are associated with a high incidence of malignancy and on the cause of the immunodeficiency associated with malignancies of the B cell or plasma cell system. In addition, these studies are directed at defining retained immunological capabilities of T cell leukemias.

Progress: The contractor has established radioimmunoassays for IgG, IgA, and IgM of man and for IgA and IgM of the mouse. These assays have been used to quantitate the rate of immunoglobulin synthesis by pokeweed mitogen stimulated, peripheral blood lymphocytes of man or of splenic lymphocytes of the mouse in in vitro cultures. Patients with a T cell leukemia associated with the Sezary syndrome have been shown to have a malignant expansion of helper T cells whereas a patient with acute lymphocytic leukemia was shown to have a malignancy of the suppressor T cells. Patients with common variable hypogammaglobulinemia have been shown to have excessive numbers of suppressor T cells that inhibit gammaglobulin synthesis by B cells. Certain patients with selective IgA deficiency have been shown to have an IgA class specific suppressor. Patients with multiple myeloma have hypogammaglobulinemia due in part to excessive numbers of suppressor macrophages. These studies are defining the nature of disorders of the immune system that lead to a high incidence of cancer. In addition, they provide information on the cause of immunodeficiency that arises secondary to certain forms of malignancy. Finally, these studies have provided insights into the functional nature of T cell leukemic cells.

Significance for Cancer Research (NCP Objective 3 Approach 4)

Project Officer: Thomas A. Waldmann, M.D.
Program: Biology Support
Technical Review Group: Ad Hoc Review Group
Relevance Review Group: DCBD Staff
FY 77 Funds: $106,000

CONTRACT RESEARCH SUMMARY

Title: Detection and Expression of Tumor Viruses

Principal Investigator: Dr. Douglas Lowy
Name/Address: Meloy Laboratories
Performing Organization: Rockville, MD

Contract Number: NO1-CP-43236
Starting Date: 11/19/76 Expiration Date: 6/15/77

Goal: To study expression of tumor viruses in tissue culture and in animals.

Approach: Viral biochemical, serologic and biological perimeters are monitored in different systems to define the regulatory mechanisms of tumor virus expression and to relate the expression to tumor production.

Progress: A quantitative assay for transfection of mammalian leukemia and sarcoma viruses has been developed for use in studies of control of viral expression.

In addition, glucocorticoids have been found to induce foci of morphologically transformed cells in a morphologically untransformed mink cell line which contains the Moloney murine sarcoma viral genome. The continued presence of glucocorticoid is required for the maintenance of foci. The induction of foci correlates with intracellular increases in sarcoma virus-specific viral RNA and in murine viral p30 protein.

Significance for Cancer Research (NCP Objective 6 Approach 2)

Project Officer: Dr. Douglas Lowy
Program:
Technical Review Group: None Site Visit Date: None
Relevance Review Group: None
FY 77 Funds: $54,000

CONTRACT RESEARCH SUMMARY

Title: Small Animal Holding Facility

Principal Investigator: Andrew S. Tegeris
Name/Address Pharmacopathics Research
Performing Organization: Laboratories, Inc.
Laurel, MD

Contract Number: NO1-CB-33887
Starting Date: 7/1/73 Expiration Date: 6/30/78

Goal: To maintain and observe animals on experiments designed to develop the means to reduce the effectiveness of external agents for producing cancer with a variety of types of materials, particularly mineral fibers and tobacco products.

Approach: Improve animal models of human cancer and evaluate effects of combined factors by assessing the resonse of the pleura of the rat to fibers and other forms of minerals that could be expected to reach this site in man.

Progress: We are currently testing carcinogenic response to many types of durable fibers in the pleura of the rat. Results suggest that fiber size is critical to carcinogenesis by asbestos and other fibers. Tobacco smoke fractions and air pollutant fractions are being evaluated for carcinogenicity on a quantitative level.

Significance for Cancer Research (NCP Objective 1 Approach 1-4)
Animal models of human cancer are essential to identifying causative agents, mechanisms of action and therapeutic trials.

Project Officer: Mearl F. Stanton, M.D.
Program: General Contract Site Vist Date: Monthly
Technical Review Group: Ad Hoc Relevance Review Group
Relevance Review Group: DCBD Contract Review Board
FY 77 Funds: $125,605

CONTRACT RESEARCH SUMMARY

Title: A Study of Phylogenetic Aspects of Neoplasia

Principal Investigator: Dr. John C. Harshbarger
Name/Address The Smithsonian Institution
Performing Washington, D.C. 20560
Organization: Registry of Tumors in Lower Animals

Contract Number: NO1-CB-33874
Starting Date: July 1, 1973 Expiration Date: June 30, 1977

Goal: To collect, examine, classify, and preserve neoplasms in cold-blooded vertebrate and invertebrate animals, and to study experimentally the development of tumors in lower animals.

Approach: The Principal Investigator directs the operation of a Registry of Tumors in Lower Animals. Specimens are acquired from personal field investigations or through submittal by other investigators. The specimens are examined grossly, histologically, and in some cases by electron microscopy. Diagnoses are established and the specimens are described. The world literature on tumors in lower animals is collected. Field investigations and experimental inductions of tumors in lower animals are carried out. Publications of the findings are made. The Registry also serves in a consulting capacity to other agencies concerned with diseases in lower animals, such as the Environmental Protection Agency, the U.S. Bureau of Fisheries (Commerce Dept.), and the Environmental Health Center.

Progress: Specimen accessions during the past year numbered 213, bringing the total collection to 1598. Forty-two percent of the total are neoplasms, the majority from teleost fish, amphibians, and reptiles. While lesions that appear to be neoplastic by morphological criteria continue to be found in molluscs and arthropods (insects alone represented), the other invertebrate phyla appear to have no representation except for lesions of controversial nature. Types of tumors accessioned are listed in the 1976 Progress Report of the Registry. The world literature collection expanded by some 200 papers, bringing the total to 3,150. Eight original reports and reviews were published by the Director during the 1976 fiscal year.

Significance for Cancer Research: Field studies and anatomical studies indicate environmental carcinogens that may be of importance in human cancer epidemiology or may be useful in designing analytical experiments to determine mechanisms of tumorigenesis.

Project Officer: Clyde J. Dawe, M.D.
Program: Cancer Biology Support Site Visit Date: May 12, 1977
Technical Review Group:
Relevance Review Group: DCBD Contract Review Board
FY 77 Funds: $119,717

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